Science
Library

126

OCT 28 1938

Volume 46 October, 1938 Number 4

SOIL SCIENCE

Editor-in-Chief
JACOB G. LIPMAN

Associate Editor
HERMINIE BROEDEL KITCHEN

Contents

The Effect of Sheep and Goat Manure on Some Mediterranean Red Soils. M. Puffeles and S. Adler 273
Economical Uses of Fertilizer Elements by Certain Forage Crops. Basil E. Gilbert and Frederick R. Pember 279
Effect of "Alkali" Salts on General Microbial Function in Soil. Walter B. Bollen and Sadegh M. Ahi 287
Further Studies of Bacterium Globiforme and the Incidence of This Type of Organism in Canadian Soils. C. B. Taylor 307
Oxidation-Reduction Potentials in Soils. Amar Nath Puri and Anand Sarup 323
A Mechanical Device for Determining the Permanent Wilting Point of Soils by Means of the Cohesion Method. George John Bouyoucos 331
On the Formation of Structure in Soil: VI. Method of Microscopic Investigation of Soil Structure in Reflected Light. D. I. Sideri ... 337

Published Monthly by THE WILLIAMS & WILKINS COMPANY
MT. ROYAL AND GUILFORD AVENUES, BALTIMORE, MARYLAND, U. S. A.

Copyright, 1938, The Williams & Wilkins Company

Made in United States of America

SOIL SCIENCE

FOUNDED BY
RUTGERS COLLEGE
NEW BRUNSWICK, N. J.

ADVISORY STAFF

A. W. Blair, J. S. Joffe, L. L. Lee, H. C. McLean, A. L. Prince, J. W. Shive, H. B. Sprague, R. L. Starkey, and S. A. Waksman, New Jersey Agricultural Experiment Station, New Brunswick, N. J.

CONSULTING EDITORS

F. J. ALWAY
University of Minnesota, St. Paul, Minn.

K. ASO
Imperial University, Tokyo, Japan

C. BARTHEL
Lantbrukshögskolan, Uppsala, Sweden

RICHARD BRADFIELD
College of Agriculture, Cornell University, Ithaca, New York

B. C. BURT
Imperial Council of Agricultural Research, New Delhi, India

H. J. CONN
New York State Experiment Station, Geneva, N. Y.

ALBERT DEMOLON
Ministry of Agriculture, Paris, France

E. B. FRED
University of Wisconsin, Madison, Wis.

V. V. GEMMERLING
Agronomitsheskii Institute, Moskovskii Universitat, Moscow, U. S. S. R.

J. E. GREAVES
Utah Agricultural College, Logan, Utah

D. J. HISSINK
Soil Science Institute, Groningen, Holland

D. R. HOAGLAND
University of California, Berkeley, California

O. LEMMERMANN
Berlin-Zehlendorf, Germany

C. B. LIPMAN
University of California, Berkeley, Calif.

BURTON E. LIVINGSTON
Johns Hopkins University, Baltimore, Md.

HENRIK LUNDEGÅRDH
Centralanstalten för jordbruksförsök, Stockholm, Sweden

M. M. MCCOOL
Boyce-Thompson Institute, Yonkers, N. Y.

W. H. MACINTIRE
University of Tennessee, Knoxville, Tenn.

S. MATTSON
Lantbrukshögskolan, Uppsala, Sweden

S. MIKLASEWSKI
Institute of Soil Science, Warsaw, Poland

E. A. MITSCHERLICH
University of Königsberg, Germany

C. A. MOOERS
University of Tennessee, Knoxville, Tenn.

D. N. PRIANISHNIKOV
Timiryazev Academy, Moscow, U. S. S. R.

THEO. REMY
Bonn-Rhein, Germany

G. W. ROBINSON
University College of Wales, Bangor, Great Britain

G. ROSSI
R. Università di Napoli, Portici, Italy

E. J. RUSSELL
Rothamsted Experimental Station, Harpenden England

O. SCHREINER
U. S. Department of Agriculture, Washington, D. C.

ALEXIUS A. J. DE'SIGMOND
Royal Hungarian Joseph University of Technical Sciences, Budapest, Hungary

N. M. TULAIKOV
Agricultural Experiment Station, Saratov, U. S. S. R.

EMILE H. DEL VILLAR
Forestry Institute, Madrid, Spain

S. WINOGRADSKY
Pasteur Institute, Paris, France

Entered as second-class matter, May 12, 1919, at the post office at Baltimore, Maryland, under the act of March 3, 1879.
Subscription price $5.00 per volume. Single numbers, $1.00

THE EFFECT OF SHEEP AND GOAT MANURE ON SOME MEDITERRANEAN RED SOILS

M. PUFFELES AND S. ADLER[1]

Government Central Laboratories, Jerusalem, Palestine

Received for publication April 13, 1938

In a previous treatise one of the authors (6) dealt with the questions of why degradation is evident in Mediterranean red-sand soils and why the formation of "Nazaz" or "Pan" increases the degradation to such an extent as to render these soils uncultivable. The present investigation shows that an addition of organic fertilizer, in the form of sheep and goat manure, to these soils is capable of improving their physical properties and thus of counteracting the degradation. The procedure in arriving at this sequel comprised the following determinations: analyses of the sheep and goat manure; analyses of the soils; analyses of mixtures of the soils and manure; determination of the effects of the mixture on the physical and mechanical properties of the soils.

ANALYSES OF SHEEP AND GOAT MANURE

Samples of goat and sheep manure from the Acre Stud Farm were examined in order to determine the differences in their chemical composition; the effect of the season on their composition; and the differences in their composition when the animals were grazed and when fed in the stall. This investigation was carried on for a year, covering the various seasons and the manifold ways in which the animals were fed. During this period eight consignments were received, each consisting of four samples, as follows: (A) sheep manure from stall, (B) sheep manure from field, (C) goat manure from stall, (D) goat manure from field.

Table 1 shows the analytical data on combined and free ammonia and total nitrogen in the fresh manure. The results were calculated on the fresh and on the moisture-free basis. On the air-dry samples, the following determinations were made: moisture, organic matter, combined NH_4, free NH_3, total nitrogen, P_2O_5, and K_2O; the ratio between organic matter and total nitrogen was also calculated. The methods of analyses adopted were as follows:

A 500-gm. portion of each sample was dried in the air until fit for crushing. A weighed portion of each of these air-dry samples was then oven-dried at 98–100°C. until constant weight was reached. The loss of moisture thus determined was calculated as percentage, and this was considered as the "moisture content of air-dry sample." Another 5-gm. portion

[1] The authors wish to express their thanks to the government analyst, G. W. Baker, for his advice and for correction of this paper.

of a mixed sample was similarly oven dried. This was considered as the "moisture-free sample."

Organic matter was determined (7) by igniting the manure and subtracting the moisture and ash, as suggested by Koenig. The accuracy of the determination was also checked by the direct method through wet oxidation.

The "free ammonia" was found by distilling 25 gm. of the fresh sample into 0.1 *N* H_2SO_4. The "combined ammonia" was found by using the same substance, adding to it 2 gm. of magnesium oxide, and distilling it again into 0.1 *N* H_2SO_4.

Total nitrogen was determined by the Kjeldahl method modified to include all the nitrates (1, p. 25).

P_2O_5 was determined according to the A.O.A.C. method (1, p. 19). K_2O was found by separating SO_4 and then by using the perchloric acid method.

TABLE 1

*Analyses of sheep and goat manure**

DESCRIPTION	FRESH SAMPLES				FRESH SAMPLES CALCULATED AS MOISTURE FREE		
	Moisture in air-dry sample	Combined NH_4	Free NH_3	Total nitrogen	Combined NH_4	Free NH_3	Total nitrogen
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
A Sheep manure from stall	58.4	0.027	0.144	0.8	0.079	0.423	2.35
B Sheep manure from field	66.6	0.030	0.058	0.58	0.123	0.237	2.37
C Goat manure from stall	60.2	0.019	0.088	0.7	0.061	0.285	2.27
D Goat manure from field	63.2	0.023	0.064	0.65	0.084	0.234	2.38

Air-dry samples calculated as moisture free

SAMPLE	MOISTURE AT 100°C.	ORGANIC MATTER	COMBINED NH_4	FREE NH_3	TOTAL NITROGEN	RATIO ORGANIC MATTER TO TOTAL N†	P_2O_5	K_2O
	per cent	*per cent*	*per cent*	*per cent*	*per cent*		*per cent*	*per cent*
A	7.6	74.2	0.021	0.051	2.0	37.1	1.83	0.77
B	9.0	73.6	0.043	0.036	2.0	36.8	1.76	0.76
C	9.0	76.2	0.022	0.025	2.1	36.3	1.56	0.68
D	9.5	75.4	0.023	0.024	2.1	35.9	1.50	0.69

* Every figure represents the average of eight determinations made on consecutive dates.
† Total nitrogen = 1.

The following conclusions may be drawn from table 1:

In drying, the organic manure loses some of its nitrogen.

More "free ammonia" is produced by feeding the animals in stalls than by grazing.

There is no marked difference between goat and sheep manure in percentages of all other constituents.

ANALYSES OF THE SOILS

In order to have a rather varied representation of soils, two samples were obtained, one from the hilly land of Rehovoth in the south of Palestine, and the other from the marshy land of Hederah in the north. The first sample

consisted of red-sand soil, poor in lime (less than 1.5 per cent); and since the profile was homogeneous, it was taken from a depth ranging between 0 and 100 cm. The second consisted of dark-red heavy soil, also poor in lime (less than 1.5 per cent), homogeneous, and from a depth of 0 to 100 cm.

Table 2 shows the results of the mechanical and physical determinations of these soils. The mechanical analyses were determined according to Beam (2); the water-holding capacity, according to Keen (4); the wilting point, according to Briggs and McLane (3). The moisture equivalent of the sample was determined by centrifuging, and then dividing the result by 1.84. After the true and the apparent specific gravities were determined, the pore space

TABLE 2

Analyses of soil samples

SAMPLE	MOISTURE AT 100°C.	MECHANICAL ANALYSIS*				PHYSICAL DETERMINATIONS					
		Clay	Silt	Fine sand	Coarse sand	Water-holding capacity	Wilting point†	True specific gravity (*a*)	Apparent specific gravity (*b*)	Pore space‡	Water permeability§
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*					
Heavy soil from Hederah.....	9.0	61.0	9.6	20.0	9.4	54.3	24.9	2.41	1.43	40.0	1,714 hours
Light soil from Rehovoth.....	4.2	22.4	2.4	20.2	55.0	37.0	16.5	2.64	1.53	42.0	67 hours, 40 min.

* According to Beam (2), Soudan method.

† Moisture equivalent. / L84

‡ $\frac{a-b}{a} \times 100$.

§ Hours per liter.

was calculated by the formula $\frac{a-b}{a} \times 100$ (where a = true specific gravity; b = apparent specific gravity). The water permeability was expressed in hours per liter. These data were carefully considered in order to show the change in the mechanicophysical properties after the addition of organic substances.

ANALYSES OF MIXTURES OF SOIL AND MANURE

The samples of manure, which did not vary greatly in composition, were combined and ground, and one part was mixed with four parts of the soil. The water optimum was considered to be the mean value between the wilting point and the water-holding capacity. The water optimum was maintained

during the experiment, the daily loss being determined, and the amount evaporated being replaced. The mixture, spread in a thin layer, was kept in the open air to ensure thorough decomposition. The carbon and the nitrogen were determined after every fortnight until constant values were obtained, and the results as moisture-free were calculated. For the calculation of carbon, the organic matter was determined, and the result was multiplied by 0.58. From the analytical data, given in table 3, the following facts are apparent:

C diminishes rapidly in the first 2½ months, whereas N changes very little in the same interval;

C decomposes gradually during the remaining 3½ months, whereas N remains constant;

At the end of 6 months, the two constituents remain almost constant.

TABLE 3

Carbon and nitrogen contents of mixtures of soils and manure

DATES, 1937	HEAVY SOIL			LIGHT SOIL		
	Carbon	Total N	Ratio* C/N	Carbon	Total N	Ratio* C/N
	per cent	*per cent*		*per cent*	*per cent*	
July 1	8.0	0.34	23.5	5.9	0.28	21.1
14	7.1	0.34	20.9	6.4	0.31	20.6
August 1	6.6	0.32	20.6	5.3	0.30	17.6
14	6.0	0.30	20.0	4.5	0.27	16.7
September 1	5.4	0.28	19.3	3.9	0.26	15.0
14	4.9	0.27	18.1	3.8	0.25	15.2
October 1	4.2	0.27	15.6	3.1	0.25	12.4
14	4.0	0.27	14.8	2.7	0.24	11.2
November 1	3.8	0.27	14.0	2.8	0.24	11.7
14	3.3	0.27	12.2	2.6	0.24	10.9
December 1	3.2	0.27	11.9	2.7	0.24	11.2
14	3.3	0.27	12.2	2.6	0.24	10.9

* Total nitrogen = 1.

EFFECTS OF MIXTURE ON PHYSICAL AND MECHANICAL PROPERTIES OF SOILS

Table 4 shows how the mechanical and physical properties of the soils have been changed as a result of the mixture with manure. Addition of organic fertilizers diminishes the clay content of heavy soils but does not appreciably change that of light soils. The following noteworthy changes occur in the physical properties:

The water-holding capacity, as well as the wilting point, rises in both the light and the heavy soils;

The pore space increases;

The water permeability becomes more advantageous in both soils. In the heavy soils, without the addition of organic substances, the time of permeability of 1 liter of water is 1,714 hours; after addition of manure, this period diminishes to 1,200 hours. In the light soils, however, the addition of organic substances prolongs the time of permeability from 67 hours and 40 minutes to 90 hours.

Table 5, essentially a comparative table, gives analytical data of exchangeable bases obtained before the addition of manure and after the addition had resulted in a constant C/N ratio. The soils were freed from the cations Ca, Mg, K, and Na by means of a 0.05 *N* HCl solution; washed with distilled water until they were free from chlorine; and dried until they became water-free. These base-free soils were then shaken for 48 hours with a solution of *N* $BaCl_2$ (1:5), filtered, washed again until they were free from chlorine, dried, and Ba-regenerated, then evaporated to a small volume, precipitated with H_2SO_4, washed, filtered, and ignited to a constant weight. The result was expressed as milliequivalents of Ba. Table 5 shows that the Ba content of the light soil

TABLE 4

Analyses of mixtures of soil and manure*

SAMPLE	MOISTURE AT 100°C.	MECHANICAL ANALYSIS				PHYSICAL DETERMINATIONS					
		Clay	Silt	Fine sand	Coarse sand	Water-holding capacity	Wilting point	True specific gravity	Apparent specific gravity	Pore space	Water permeability
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*					*hours*
Heavy soil from Hederah....	7.9	49.4	14.0	19.6	17.0	75.9	34.6	1.85	1.00	45.0	1,200
Light soil from Rehovoth....	3.2	22.4	6.4	16.6	54.6	63.0	29.1	1.93	1.03	46.0	90

* See footnotes to table 2.

TABLE 5

Exchangeable bases in soils before and after addition of manure

DESCRIPTION AND SOURCE	Ba
	m.e.
Light soil from Rehovoth without manure	6.6
Light soil from Rehovoth with organic manure	14.6
Heavy soil from Hederah without manure	28.5
Heavy soil from Hederah with organic manure	38.5

increases from 6.6 to 14.6 m.e.; and that of the heavy soil, from 28.5 to 38.5 m.e. These figures agree with the values arrived at by McGeorge (5) and Turner (8), who concluded that for 10 gm. of C in the soil, the exchangeability increases by 35 m.e.; according to our results it increases by 30.3 m.e. in heavy soils and 30.7 m.e. in light soils.

CONCLUSIONS

The analyses of the sheep and goat manure show that the composition is not materially affected by either the seasons or by the variation and manner of feeding. On drying the manure, some loss of nitrogen is observed.

The decomposition of the manure, as indicated by the C/N ratio, appears to have been completed in 6 months, under laboratory conditions in which the soil was kept at constant moisture content.

The C/N ratio of "humus-like" substances and the increase of the value of exchangeable bases as a result of the proportional increase of C are similar to results obtained by other investigators.

The addition of organic substances not only enhances the supply of such nutritives as K, N, and P_2O_5 to the soils and improves the physical properties, in general, but increases also the exchangeable bases.

REFERENCES

(1) Association Official Agricultural Chemists 1935 Tentative and Official Methods of Analysis, ed. 4. Washington, D. C.

(2) Beam, W. 1911 The mechanical analysis of arid soils. *Cairo Sci. Jour.* 5: 107–109.

(3) Briggs, L. J., and McLane, J. W. 1910 Moisture equivalent determinations and their application. *Proc. Amer. Soc. Agron.* 2: 138–147.

(4) Keen, B. A. 1931 The Physical Properties of the Soil. Longmans, Green and Co., New York.

(5) McGeorge, W. T. 1936 Base exchange properties of organic matter in soils. Ariz. Agr. Exp. Sta. Tech. Bul. 30.

(6) Puffeles, M. 1937 Some data on the Mediterranean red soils. *Soil Sci.* 44: 167–173.

(7) Schollenberger, C. J. 1927 A rapid approximate method for determining soil organic matter. *Soil Sci.* 24: 65–68.

(8) Turner, P. E. 1932 An analysis of factors contributing to the determination of saturation capacity in some tropical soil types. *Jour. Agr. Sci.* 22: 72–91.

ECONOMICAL USES OF FERTILIZER ELEMENTS BY CERTAIN FORAGE CROPS[1]

BASIL E. GILBERT AND FREDERICK R. PEMBER[2]

Rhode Island Agricultural Experiment Station

Received for publication April 23, 1938

Earlier workers (1, 3) of the Rhode Island Agricultural Experiment Station investigated the fertilizer nutrient requirements of cereals grown in solution and soil cultures in the greenhouse. Minimum percentages of nitrogen, phosphorus, and potassium which brought about satisfactory growth and yields of grain and straw by barley, oats, and wheat were established. With the increased interest developing in the northeastern dairy regions of the United States in pasture improvement problems, the emphasis in research shifts from cereals to forage crops, and the need of more definite knowledge as to methods of stimulating the growth of pasture grasses is apparent. Little or no work, comparable to the experiments with cereals, seems to have been done with redtop, timothy, or colonial bent.

GROWTH METHODS

Essentially the same technic as that used by former workers at this station with cereals was followed in a study of the nutrient requirements of timothy, redtop, and colonial bent (R. I. Bent). This technic, established by Pember (3), has been described in detail by Pember and McLean (4). It is unnecessary, therefore, to go into detail in the description of the growth methods followed, except in those points where minor changes were made.

In the previous work with cereals, considerable care was taken to obtain yields of grain. As the vegetative portions of the forage crops are of greatest value, dates of harvesting were chosen, with both solution and soil cultures, to correspond with the maximum vegetative yields of the crops studied.

Solution cultures

Each culture contained only 10 plants and was grown in triplicate. The seedlings were carefully selected as to uniformity and were grown in nutrient solution (4) from January to May of each year. The fertilizer nutrients were varied in each culture solution in an endeavor to produce optimum and suboptimum conditions.

[1] Published, by permission of the director of research, as Contribution No. 526 of the Rhode Island Agricultural Experiment Station.

[2] Deceased; formerly associate plant physiologist.

In order to safeguard against the possibility of limiting conditions due to very low concentrations of the minor elements, at each change of solution small amounts of magnesium sulfate, ferric nitrate, manganese sulfate, and sodium borate [4 cc. 0.1 *M* $MgSO_4$, 1.18 gm. per liter of Fe $(NO_3)_3$, 1 p.p.m. Mn, and 1 p.p.m. B] were added. The tillers were clipped from all cultures weekly and were dried and weighed separately from the tops and roots.

Soil cultures

Timothy, redtop, and colonial bent were grown in soil cultures in Wagner pots with varying amounts of K_2O, N, and P_2O_5 applied in fertilizer salts. In 1931 and 1932 the soil used for the potash series was taken from plat 116 of the agronomy farm. This plat is considered to be very low in residual potash. For the nitrogen series, the soil from plat 116 was used in 1931; and then, since there may have been some nitrogenous residue from organic matter, soil from a fallow area was used in 1932. For the phosphorus series in both years soil from plat 67, which is the low-phosphorus plat of the phosphate series of agronomy plats, was used.

All the P_2O_5 was applied as superphosphate (21.7 per cent P_2O_5) at the beginning of the experiments. The K_2O was applied as K_2SO_4 (49.9 per cent K_2O) at intervals during the growth of the cultures, and the nitrogen was also applied at intervals as $NaNO_3$ (16.2 per cent N), the first application of each being made when the plants were transplanted to the pots.

After being harvested, the plants were air-dried and weighed.

PRESENTATION OF DATA

Though several workers have studied the response to fertilizer nutrients of redtop and timothy when grown under field conditions, few attempts have been made to study either these forage crops or colonial bent under such controlled conditions as can be secured in solution or soil cultures under glass. The dry weights of the cultures grown in these experiments show some interesting results.

Solution cultures

Table 1 presents the total nutrients added, in grams per culture, and the dry weight yields of roots, tops, and clips of timothy, redtop, and colonial bent grasses grown in culture solutions. The average relative yields are included so that it is possible to determine readily the relationships which may exist between fertilizer nutrients and yields.

It will be noted that all crops responded most to potash insofar as roots were concerned. The tops of colonial bent and redtop were affected most by the extra amounts of nitrogen. Timothy tops seemed to benefit most from the increased quantities of potash in the solution. When the clippings for all crops were considered, the low-nitrogen cultures gave the lowest weights.

TABLE 1

Nutrients added and dry weight yields of timothy, redtop, and colonial bent grown in culture solutions

	NUTRIENTS ADDED, PER CULTURE			DRY WEIGHT YIELDS			AVERAGE RELATIVE YIELDS FOR TWO CROPS			
	N	P_2O_5	K_2O	Roots	Tops	Clips	Roots	Tops	Clips	Tops and clips
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*				
Colonial bent										
1931										
Complete NPK	.269	.091	.199	7.4	11.7	3.9	100	100	100	100
Low N	.061	.091	.199	5.4	4.9	2.0	78	45	48	46
Low P	.269	.017	.199	4.9	5.4	2.3	71	52	59	54
Low K	.269	.091	.042	3.3	8.4	2.9	38	62	70	65
1935										
Complete NPK	.280	.116	.216	5.6	8.8	4.4				
Low N	.076	.116	.216	4.8	4.4	2.0				
Low P	.280	.021	.216	4.3	5.3	2.6				
Low K	.280	.116	.036	1.7	4.4	2.9				
Timothy										
1931										
Complete NPK	.398	.128	.268	11.5	19.2	5.2	100	100	100	100
Low N	.116	.128	.268	9.0	15.3	2.3	83	72	43	69
Low P	.398	.020	.268	8.1	10.0	2.2	71	67	41	63
Low K	.398	.128	.047	3.2	7.2	5.1	37	48	94	54
1935										
Complete NPK	.304	.122	.234	7.9	30.2	1.7				
Low N	.088	.122	.234	7.1	20.3	0.7				
Low P	.304	.021	.234	5.7	22.9	0.6				
Low K	.304	.122	.036	4.0	16.6	1.4				
Redtop										
1931										
Complete NPK	.271	.091	.194	9.5	9.8	5.4	100	100	100	100
Low N	.071	.091	.194	5.9	3.8	2.5	69	46	52	48
Low P	.271	.021	.194	6.9	5.8	2.8	75	61	49	57
Low K	.271	.091	.042	2.7	4.3	4.5	35	50	83	61
1935										
Complete NPK	.312	.125	.240	8.6	9.2	3.8				
Low N	.092	.125	.240	6.5	5.0	2.3				
Low P	.312	.021	.240	6.6	5.8	1.7				
Low K	.312	.125	.036	3.6	5.2	3.1				

When weights of tops and clips were averaged, the greatest response was obtained with nitrogen in the cultures of colonial bent and red top, and with potash in the cultures of timothy. This reaction of timothy to potash is of

TABLE 2

Nutrients added and dry weight yields of timothy, redtop, and colonial bent grown in soil cultures

	NUTRIENTS ADDED, PER CULTURE			DRY WEIGHT YIELDS			AVERAGE RELATIVE YIELDS		
	N	P_2O_5	K_2O	Tops	Clips	Total	Tops	Clips	Total
	gm.	gm.	gm.	gm.	gm.	gm.			
Redtop									
1931									
No N.....	.0	1.085	1.248	1.3	1.5	2.8	22	18	20
1 N.....	.081	1.085	1.248	1.8	2.8	4.6	30	34	32
2 N.....	.162	1.085	1.248	2.8	3.9	6.7	47	47	47
3 N.....	.405	1.085	1.248	6.0	8.3	14.3	100	100	100
No P.....	.405	0.0	1.248	6.3	5.5	11.8	105	66	83
1 P.....	.405	0.217	1.248	4.8	6.4	11.2	80	77	78
2 P.....	.405	0.434	1.248	5.5	6.8	12.3	92	82	86
3 P.....	.405	1.085	1.248	6.0	8.3	14.3	100	100	100
No K.....	.405	1.085	0.0	5.5	6.6	12.1	115	96	103
1 K.....	.405	1.085	1.250	5.0	6.8	11.8	104	99	101
2 K.....	.405	1.085	0.998	4.5	6.5	11.0	94	94	94
3 K.....	.405	1.085	1.248	4.8	6.9	11.7	100	100	100
1932									
No N.....	.0	1.092	1.440	1.5	2.8	4.3	21	34	28
1 N.....	.081	1.092	1.440	3.8	3.8	7.6	54	46	50
2 N.....	.162	1.092	1.440	6.5	4.9	11.4	93	60	75
3 N.....	.324	1.092	1.440	7.0	8.2	15.2	100	100	100
No P.....	.324	0.0	1.440	5.0	4.0	9.0	125	50	75
1 P.....	.324	0.219	1.440	5.8	7.5	13.3	145	94	111
2 P.....	.324	0.437	1.440	8.0	7.6	15.6	200	95	130
3 P.....	.324	1.092	1.440	4.0	8.0	12.0	100	100	100
No K.....	.324	1.092	0.0	5.0	8.6	13.6	116	100	105
1 K.....	.324	1.092	0.240	7.5	7.9	15.4	174	92	119
2 K.....	.324	1.092	0.960	9.4	6.9	16.3	219	80	126
3 K.....	.324	1.092	1.440	4.3	8.6	12.9	100	100	100
Colonial bent									
1931									
No N.....	.0	1.085	1.248	2.0	1.5	3.5	35	23	29
1 N.....	.081	1.085	1.248	4.5	2.2	6.7	78	34	55
2 N.....	.162	1.085	1.248	6.3	3.3	9.6	109	52	79
3 N.....	.405	1.085	1.248	5.8	6.4	12.2	100	100	100
No P.....	.405	0.0	1.248	2.0	3.0	5.0	34	47	41
1 P.....	.405	0.217	1.248	4.0	4.8	8.8	69	75	72
2 P.....	.405	0.434	1.248	2.8	4.4	7.2	48	69	59
3 P.....	.405	1.085	1.248	5.8	6.4	12.2	100	100	100
No K.....	.405	1.085	0.0	10.0	6.0	16.0	102	120	108
1 K.....	.405	1.085	0.250	6.0	5.2	11.2	61	104	76
2 K.....	.405	1.085	0.998	7.8	5.3	13.1	80	106	89
3 K.....	.405	1.085	1.248	9.8	5.0	14.8	100	100	100

TABLE 2—*Concluded*

	NUTRIENTS ADDED, PER CULTURE			DRY WEIGHT YIELDS			AVERAGE RELATIVE YIELDS		
	N	P_2O_5	K_2O	Tops	Clips	Total	Tops	Clips	Total
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*			
Colonial bent—Continued									
1932									
No N.....	.0	1.092	1.440	4.0	1.4	5.4	36	25	32
1 N.....	.081	1.092	1.440	6.5	2.1	8.6	59	37	51
2 N.....	.162	1.092	1.440	9.5	3.1	12.6	86	54	75
3 N.....	.324	1.092	1.440	11.0	5.7	16.7	100	100	100
No P.....	.324	0.0	1.440	4.0	3.2	7.2	38	64	46
1 P.....	.324	0.219	1.440	12.0	3.9	15.9	114	78	103
2 P.....	.324	0.437	1.440	8.5	3.9	12.4	81	78	80
3 P.....	.324	1.092	1.440	10.5	5.0	15.5	100	100	100
No K.....	.324	1.092	0.0	8.5	5.3	13.8	85	82	84
1 K.....	.324	1.092	0.240	10.8	4.7	15.5	108	72	94
2 K.....	.324	1.092	0.960	11.5	4.9	16.4	115	75	99
3 K.....	.324	1.092	1.440	10.0	6.5	16.5	100	100	100
Timothy									
1931									
No N.....	.0	1.085	1.248	3.0	2.2	5.2	14	38	20
1 N.....	.081	1.085	1.248	4.3	3.5	7.8	21	60	30
2 N.....	.162	1.085	1.248	8.8	4.5	13.3	43	78	51
3 N.....	.405	1.085	1.248	20.3	5.8	26.1	100	100	100
No P.....	.405	0.0	1.248	8.8	0.7	9.5	43	12	36
1 P.....	.405	0.217	1.248	16.8	3.3	20.1	83	57	77
2 P.....	.405	0.437	1.248	22.8	4.6	27.4	112	79	105
3 P.....	.405	1.085	1.248	20.3	5.8	26.1	100	100	100
No K.....	.405	1.085	0.0	20.3	6.5	26.8	108	90	103
1 K.....	.405	1.085	0.250	18.8	6.7	25.5	100	93	98
2 K.....	.405	1.085	0.998	18.5	7.2	25.7	98	100	99
3 K.....	.405	1.085	1.248	18.8	7.2	26.0	100	100	100
1932									
No N.....	.0	1.092	1.440	3.8	1.4	5.2	17	15	17
1 N.....	.081	1.092	1.440	8.0	3.0	11.0	37	32	35
2 N.....	.162	1.092	1.440	11.5	4.1	15.6	53	44	50
3 N.....	.486	1.092	1.440	21.8	9.4	31.2	100	100	100
No P.....	.486	0.0	1.440	12.0	4.7	16.7	73	53	66
1 P.....	.486	0.219	1.440	15.0	6.7	21.7	91	76	86
2 P.....	.486	0.437	1.440	13.0	8.0	21.0	79	91	83
3 P.....	.486	1.092	1.440	16.5	8.8	25.3	100	100	100
No K.....	.486	1.092	0.0	21.3	6.8	28.1	164	79	130
1 K.....	.486	1.092	0.240	17.8	7.1	24.9	137	83	115
2 K.....	.486	1.092	0.960	17.0	8.4	25.4	131	98	118
3 K.....	.486	1.092	1.440	13.0	8.6	21.6	100	100	100

interest because it seems to be at variance with the usual concept of the responses of grasses to fertilizer elements. It should be recognized that the prevailing knowledge is based on fertilizer trials under field conditions. The data presented here may be considered as picturing more nearly the absolute relative minimum requirements of these three crops. The amounts of nutrients available to the plant in a culture solution are, in many instances, relatively less than those in fertilized soil, and in this set of experiments these amounts were planned to approximate starvation levels.

Soil cultures

In the light of the foregoing results, it is of interest to compare timothy, redtop, and colonial bent when growing in soil and with additional nutrients supplied at varying levels. Table 2 gives the nutrients applied and the dry weight and average relative yields of these three forage crops when grown under such conditions.

It will be noted that the response of all three crops, in tops, clips, and total yields, was greatest to nitrogen. Response to phosphorus, although evident, was not so marked or regular as response to nitrogen. There were indications of but slight response to potash, even with timothy, which responded markedly in the solution cultures. These results are similar to those obtained by North and Odland (2) in a study of the seed yields of colonial bent as affected by fertilization under field conditions. They note that "Phosphorus and potash produced little effect on the growth . . . ."

These results with soil cultures seem to show that, even under what would be considered low nutrient conditions, timothy, redtop, and colonial bent are able to produce optimum growth, provided sufficient nitrogen is supplied. They also seem to show that, though conditions in the solution cultures so closely approached starvation levels for potash and phosphorus that growth was reduced, such conditions were not approached in these soil cultures.

It should be noted that these experiments were conducted in pots with one soil type only and under conditions which may not have been entirely comparable to field conditions. Other investigators have obtained results which seem to show that both phosphate and potash are helpful in stimulating vegetative growth of grasses in the field, particularly when used in conjunction with lime. No attention was given in these experiments to the importance of the composition of the forage and to its nutritive value, which under field conditions would be greatly increased by applications of phosphoric acid and potash.

SUMMARY

This study reports the comparative dry weight yields of cultures of timothy, colonial bent, and redtop, grown in solution and soil cultures with varying levels of fertilizer elements.

Solution culture results. With all the crops, root growth was increased by greater amounts of potash. The clippings or tiller growth was stimulated by

extra nitrogen. The weight of tops of colonial bent and of redtop was greatest with the higher amounts of nitrogen, and that of timothy tops was greatest with extra potash. The lowest culture conditions were near the absolute mineral nutrient requirements of these crops.

Soil culture results. All three crops responded best, in tops, clips, and total yields, to the higher nitrogen applications. The response to phosphorus was less marked than the response to nitrogen, and but slight indications of response to potash were obtained with any of the crops grown on one soil type only. It is concluded that colonial bent and redtop require less potash and phosphorus for good growth than does timothy and that all three crops require high fertilization with nitrogen.

REFERENCES

(1) HARTWELL, B. L., AND PEMBER, F. R. 1922 The feeding power of certain cereals, and their response to fertilizer ingredients. R. I. Agr. Exp. Sta. Bul. 190.

(2) NORTH, H. F. A., AND ODLAND, T. E. 1934 Seed yields of Rhode Island Colonial Bent (*Agrostis tenuis* sibth) as influenced by the kind of fertilizer applied. *Jour. Amer. Soc. Agron.* 26: 939–945.

(3) PEMBER, F. R. 1917 Studies by means of both pot and solution culture of the phosphorus and potassium requirements of the barley plant during its different periods of growth. R. I. Agr. Exp. Sta. Bul. 169.

(4) PEMBER, F. R., AND MCLEAN, F. T. 1925 Economical use of nitrogen, phosphorus and potassium by barley, oats and wheat in solution cultures. R. I. Agr. Exp. Sta. Bul. 199.

EFFECT OF "ALKALI" SALTS ON GENERAL MICROBIAL FUNCTION IN SOIL

WALTER B. BOLLEN[1] AND SADEGH M. AHI[2]

Oregon Agricultural Experiment Station

Received for publication April 19, 1938

Soil bacteria, as well as higher plants, are affected by so-called "alkali" salts; this has been recognized since the pioneer work of C. B. Lipman (11). The problems of reclamation of natural saline soils and the prevention of salinization of soils now in cultivation have led to many investigations on the effects of "alkali" salts on biological as well as physical and chemical properties of the soil.

HISTORICAL

In studies on ammonification, Lipman (10, 12) demonstrated relatively high toxicity of sodium sulfate and stimulation by sodium carbonate; he also found "antogonism" or reduction in toxicity with combinations of sodium, potassium, and magnesium cholorides, and with chloride, sulfate, and carbonate of sodium. From laboratory and greenhouse tests on a normal soil and on a saline soil, Brown and Hitchcok (2) concluded that nitrification and crop growth are similarly affected by "alkali" salts, low concentrations being stimulating, and high concentrations, with the exception of calcium carbonate, being depressive. Brown and Johnson (3) found that ammonification was markedly stimulated by 0.6 per cent calcium carbonate but was inhibited by 0.1 per cent sodium bicarbonate, 0.2 per cent sodium carbonate, and 0.5 per cent sodium sulfate.

In a series of studies by Greaves (6, 8), soil texture and moisture were found to influence the relative toxicity of various salts. Greater toxicity was exerted in a sandy soil than in a loam soil, and toxicity decreased as moisture increased. Small quantities of salts added to a soil increased bacterial activities, as indicated by increased ammonification, nitrification, and nitrogen fixation (7). Toxicity of the various salts was found closely proportional to the osmotic pressure produced in the soil, showing that toxicity is partly due to osmotic disturbance. Another factor advanced as preventing bacteria from performing their natural functions is the physiological influence of a given salt; this is determined not alone by the concentration of salt in the soil, but also by cell permeability and the physical-chemical influence of the salt upon

[1] Associate bacteriologist.

[2] Graduate student in soils.

the protoplasm. Nitrifiers were found to be more sensitive than ammonifiers, whereas the nonsymbiotic nitrogen-fixers were found to be more resistant, tolerating higher concentrations of "alkali" and other salts than do most higher plants.

Comparing ammonification, nitrification, and crop yield during production of two crops of wheat with percentage recovery of added salts in a neutral silt loam soil, Gibbs, Batchelor, and Magnuson (5) found a general tendency for any toxic action to disappear as time progressed. This was in line with the gradual decrease in total water-soluble salts; however, neither toxic nor stimulating action was proportional to total salt recovery. Although sodium carbonate was toxic to plant growth, it stimulated ammonification of blood and nitrification of ammonium sulfate; in general, sodium chloride had the reverse influence, and sodium sulfate had only slight effect.

OBJECT OF INVESTIGATION

These previous investigations have been concerned with specific groups of soil bacteria; so far as is known, similar studies on the more generalized function of organic decomposition and carbon dioxide production have not been reported. This phase of the alkali problem was attacked through the study of decomposition of native and incorporated organic matter in an acid soil treated with "alkali" salts, and in a virgin saline soil and its partially reclaimed counterpart.

METHODS

Carbon dioxide evolution during incubation at room temperature and optimum moisture content in a respiration apparatus similar to that described by Potter and Snyder (14) was used as an index of general microbial activity and organic decomposition. In the apparatus used, however, the CO_2-free air was passed over the soil by slight pressure rather than by suction, and in this way the pressure over the soil, as well as over the absorbing solution, was maintained at approximately one atmosphere. The CO_2 evolved was absorbed in approximately *N* NaOH and determined by double titration, phenolphthalein and brom-phenol-blue being used as indicators. Results are expressed as milligrams of carbon evolved as CO_2 per kilogram of soil on the water-free basis. Values for the check or untreated soil were subtracted from values for the treated soils in the plotting of all results. The curve for the check is thus plotted as the x-axis. This not only reduces the size of the graph but also presents the data in more striking form.

Chemical and microbial analyses were also made to determine changes in water-soluble anions and in pH, and in numbers of molds, bacteria, actinomyces, and *Azotobacter* cells. A 1:5 suspension of the soil was prepared with sterile distilled water and shaken for 30 minutes; the coarser particles were then allowed to settle, and a 1-cc. sample was withdrawn for the preparation

of dilutions for plate counts. Determination of pH, by means of circulating hydrogen electrode (1), was made on another portion. The remaining suspension was then filtered through a Pasteur-Chamberland candle, and the filtrate was analyzed. Association of Official Agricultural Chemists methods of analysis for waters were used for the determination of nitrate, nitrite, chloride, carbonate, and bicarbonate. Sulfate was determined by the turbidity method of Schreiner and Failyer (16). Truog and Meyer's modification (17) of the Deniges method for phosphate was used.

In the microbial counts, peptone-glucose-acid agar was used for molds, and sodium albuminate agar as described by Fred and Waksman (4) was used for bacteria and actinomyces. Duplicate plates from appropriate dilutions were poured in 150-mm. petri dishes; the number of colonies counted after incubation at 28°C. ranged from approximately 50 to 250. Numerous control plates, amounting to at least 10 per cent of the total poured, were used. *Azotobacter* was determined by the soil-paste plaque method, 5 per cent starch and 1 per cent powdered $CaCO_3$ being used.

EFFECTS OF ADDED "ALKALI" SALTS

To determine the effects of added "alkali" salts on the microbial population and its activities, the following experiment was made with Willamette silty clay loam. This is nonsaline slightly acid soil of great agricultural importance in the subhumid Willamette Valley region. A bulk sample, taken along a fence and believed to be essentially "virgin," was passed through a 2-mm. sieve and mixed. The moisture content after mixing was 21 per cent. Saturation capacity, as determined by the amount of water held against gravity by a portion saturated in a gooch and allowed to drain in a saturated atmosphere, was 52 per cent. Kjeldahl nitrogen was 0.260 per cent, and organic matter by Rather's (15) method, 4.01 per cent.

One-kilogram portions were treated with "alkali" salts alone, in combination with ground wheat straw, and in combination with wheat straw plus sodium nitrate, as indicated in figure 1. The 2 per cent concentration of salts added is excessive from the standpoint of plant tolerance, as well as for certain soil microbial functions, as has been determined by previous investigators. This concentration was used, however, in order to obtain a maximum effect on the more generalized activities which may be expected to persist even in soils containing still higher concentrations. Sodium nitrate, added in small amount with the straw in several treatments, was used in order to avoid lack of available nitrogen as a factor retarding decomposition.

Each treated portion of soil was placed in a 2.5-liter bottle, distilled water was added to bring the soil moisture to 60 per cent of the saturation capacity, and the bottle was then connected to the respiration apparatus. Carbon dioxide evolution was determined during 164 days, titrations being made at daily intervals during the first 10 days, after which longer and longer intervals were used until the final determination represented a 30-day period. Chemical

and microbial analyses were made on the original sample at the beginning of the experiment and on the check and treated portions at the close of the experiment.

The effect of the various treatments on production of carbon dioxide is illustrated in figure 1, the check being taken as zero at all times and plotted as the x-axis. Inspection of the graph reveals that the added salts depressed carbon dioxide evolution from the native soil organic matter as well as from organic matter added as wheat straw. Sodium chloride caused the most depressive effect, a marked depression below the check being obtained even with straw and straw plus nitrate. Magnesium sulfate and sodium sulfate had less depressive influence, and, like sodium chloride, showed greater effect on straw than on the native organic matter.

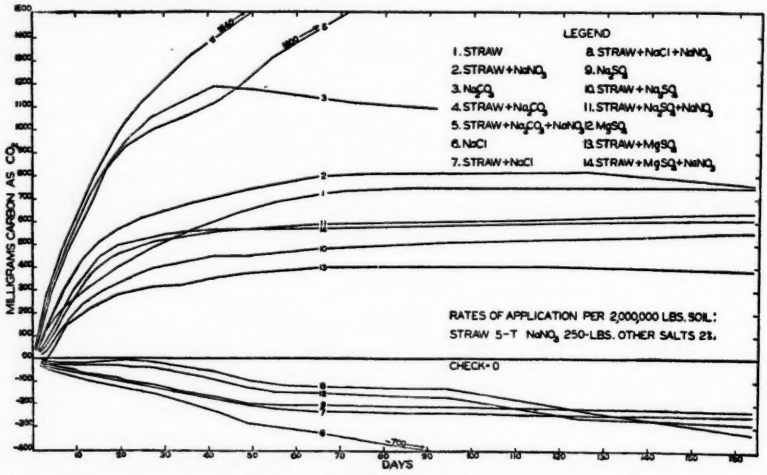


FIG. 1. EFFECT OF "ALKALI" SALTS ON ORGANIC DECOMPOSITION IN WILLAMETTE SILTY CLAY LOAM

Sodium carbonate treatment caused a large increase in CO_2 production, the greatest amount and most rapid evolution being obtained in combination with straw. This can be attributed chiefly to interaction with the acid soil and not to organic decomposition. At the close of the experiment the straw addition had resulted in an increase of 755 mgm. C as CO_2 over the check, whereas straw plus carbonate gave an increase of only 588 mgm. Since bicarbonate present represents CO_2 absorbed it also must be considered. As shown in table 1, the little difference in bicarbonate between the soil treated with sodium carbonate plus straw and sodium carbonate alone (32 p.p.m.) is almost balanced by the difference in normal carbonate (20 p.p.m.). The same is true in the case of straw and sodium carbonate plus nitrate. It is evident, therefore, that the added carbonate subsequently evolved as CO_2 is almost identical

whether added alone or in various combinations. Sodium carbonate thus retarded decomposition of the straw in all cases, its effect being intermediate as compared to sodium sulfate and magnesium sulfate. That it also retarded decomposition of the native organic matter is indicated by the latter portion of curve 3, figure 1, which shows a negative slope.

Sodium nitrate slightly increased the decomposition of straw, except in combination with sodium carbonate. It is hardly probable that the sodium concentration may have been critical, so that the 34 p.p.m. from the nitrate, added to the 8,680 p.p.m. supplied by the carbonate, produced the marked decrease in CO_2 evolution. Although there is ample evidence that nitrate additions increase decomposition of organic matter of wide carbon-nitrogen ratio, it has been occasionally observed that inorganic nitrogen compounds decrease CO_2 evolution. A stimulation of autotrophes and their assimilation

TABLE 1

Carbon balance in carbonate-treated Willamette silty clay loam

After 164 days' respiration

TREATMENT	C ADDED			C AS CO_3	C AS HCO_3	C EVOLVED FROM SOIL AS CO_2				C OF STRAW EVOLVED AS CO_2
	CO_2	Straw	Total			Total	From additions	From additions plus C as HCO_3	From straw	
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*per cent*
Check	0	0	0	0	5	1,250				
Straw	0	2,500	2,500	0	10	2,000	750	760	755	30
Na_2CO_3	2,260	0	2,260	160	702	2,280	1,030	1,732		
Straw + Na_2CO_3	2,260	2,500	4,760	180	670	2,890	1,640	2,310	578	23
Straw + Na_2CO_3 + $NaNO_3$	2,260	2,500	4,760	190	670	2,755	1,505	2,175	443	18

of CO_2 may account for this; a discussion of this point in connection with other data will be presented in a later paper. It is significant that in all cases added nitrate was apparently assimilated, the amounts recovered at the close of the experiment being the same as or less than those in soil correspondingly treated but without nitrate addition.

Results of analyses of water extracts made at the close of the experiment are given in table 2. Nitrates are significantly low in every salt-treated sample, especially where carbonate and chloride were added. Magnesium sulfate and sodium sulfate had less effect, in the order named. These results correlate closely with the trend of CO_2 evolution as influenced by the same salts; thus nitrates were higher in the presence of sodium sulfate, which was least depressive to CO_2 production. This relation, however, does not necessarily indicate direct influence on nitrification, since after greater decomposition more nitrates should appear. Moreover, the nitrate concentration was virtually the same

in all samples treated with a given salt, regardless of straw or nitrate additions, and since salt additions in all cases caused a marked decrease in nitrates, either nitrate assimilation or denitrification was stimulated. Denitrification was unlikely, because aerobiosis was maintained and because the nitrite concentration was low in all cases.

Reduction in phosphate concentration occurred in all the salt-treated soils and corresponded closely with pH changes, sodium carbonate exerting the greatest effect. Although the actual amount of water-soluble phosphate is low, even in the untreated soil, it is indicative of at least an immediately ample supply of available phosphorus.

TABLE 2

Effect of treatments on composition of water extract from Willamette silty clay loam

After 160 days' incubation

TREATMENT	N AS NO_3	N AS NO_2	P AS PO_4	Cl	S AS SO_4	C AS CO_3	C AS HCO_3	pH
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	
Original sample	30.0	0.1	2.0	Trace	2	0	3	6.20
0. No treatment	75.0	0.3	3.3	"	30	0	5	6.33
1. Straw—5 T.*	75.0	0.4	3.3	"	29	0	10	6.54
2. Straw—5 T., $NaNO_3$—125 p.p.m.	60.0	0.3	2.5	"	29	0	7	6 72
3. Na_2CO_3—2 per cent	2.5	0.7	1.5	"	30	180	702	8.43
4. Na_2CO_3—2 per cent, straw—5 T.	2.0	0.9	1.5	"	30	180	670	8.52
5. Na_2CO_3—2 per cent, straw—5 T., $NaNO_3$—125 p.p.m.	2.0	0.6	1.6	"	30	190	670	8.52
6. NaCl—2 per cent	2.5	0.6	1.7	10,600	30	0	7	7.32
7. NaCl—2 per cent, straw—5 T.	2.5	2.5	2.0	10,800	30	0	12	7.43
8. NaCl—2 per cent, straw—5 T., $NaNO_3$—125 p.p.m.	2.5	1.8	2.0	10,800	30	0	10	7.43
9. Na_2SO_4—2 per cent	10.0	0.5	2.5	Trace	4,890	0	12	7.31
10. Na_2SO_4—2 per cent, straw—5 T.	25.0	0.8	2.5	"	3,930	0	12	7.33
11. Na_2SO_4—2 per cent, straw—5 T., $NaNO_3$—125 p.p.m.	10.0	0.8	2.5	"	4,740	0	10	7.33
12. $MgSO_4$—2 per cent	5.0	0.5	2.0	"	4,825	0	12	7.41
13. $MgSO_4$—2 per cent, straw—5 T.	5.0	0.5	2.0	"	4,740	0	12	7.41
14. $MgSO_4$—2 per cent, straw—5 T., $NaNO_3$—125 p.p.m.	5.0	0.6	2.0	"	4,580	0	12	7.41

* Tons per 2,000,000 pounds soil.

The data for chlorides indicate an approximately 90 per cent recovery from added sodium chloride. Virtually all the sulfur added as sodium sulfate was recovered, and the magnesium sulfate yielded 90 per cent. This is greater recovery than that reported for a comparable period of time by Neidig and Magnuson (13) for smaller additions to Boise silt loam.

Sulfate concentration was not influenced by any treatment except sulfate additions. The otherwise general accumulation of about 30 p.p.m. of sulfur as sulfate during the incubation period indicates a normal sulfur-oxidizing

activity, even in the presence of added carbonate and chloride. The substrate originated apparently entirely from the native organic matter, decomposition of the added straw rendering inappreciable amounts of sulfur available to the sulfur-oxidizing microflora.

Microorganisms, particularly molds, were strikingly influenced by the different salt treatments, as may be seen from table 3. Total numbers in almost every case are lower than in the original sample at the beginning of the ex-

TABLE 3

Effect of treatments on microbial population in Willamette silty clay loam

After 164 days' incubation

TREATMENT	MOLDS				BACTERIA AND ACTINOMYCES		
	Total	Mucors	Penicillia	Aspergilli	Total	Actinomyces	Azotobacter*
	thousands	*per cent*	*per cent*	*per cent*	*millions*	*per cent*	
Original sample	121	2.2	88	0	5.9	42	XX
0. No treatment	136	0.4	61	0.2	2.1	14	X
1. Straw—5 T.†	57	0.3	60	0.5	2.5	8	X
2. Straw—5 T., $NaNO_3$—125 p.p.m.	80	1.2	90	0.3	1.9	11	O
3. Na_2CO_3—2 per cent	7	0	13	11.0	1.1	18	O
4. Na_2CO_3—2 per cent, straw—5 T.	2	0	17	16.6	1.4	21	O
5. Na_2CO_3—2 per cent, straw—5 T., $NaNO_3$—125 p.p.m.	18	0	14	2.9	1.2	33	O
6. NaCl—2 per cent	103	0	88	11.6	2.9	10	O
7. NaCl—2 per cent, straw—5 T.	92	0	45	9.0	2.1	5	O
8. NaCl—2 per cent, straw—5 T., $NaNO_3$—125 p.p.m.	225	0	40	3.8	4.9	6	O
9. Na_2SO_4—2 per cent	16	0	50	4.8	3.3	6	M
10. Na_2SO_4—2 per cent, straw—5 T.	47	0	43	1.0	3.6	8	M
11. Na_2SO_4—2 per cent, straw—5 T., $NaNO_3$—125 p.p.m.	23	0	88	1.1	4.7	2	M
12. $MgSO_4$—2 per cent	52	0	90	0.5	1.8	11	X
13. $MgSO_4$—2 per cent, straw—5 T.	48	0	52	0	1.5	20	X
14. $MgSO_4$—2 per cent, straw—5 T., $NaNO_3$—125 p.p.m.	130	0	64	0	1.9	11	X

* O = no development, X = moderate development, XX = heavy development, M = moldy.

† Tons per 2,000,000 pounds soil.

periment. This is to be expected, since during more than 5 months' incubation the various treatments had opportunity to react, and the microbial activities would probably decline to a lower state of relative equilibrium in all cases by the end of the period. The reduction in numbers of molds and bacteria by sodium carbonate, however, is outstanding; sodium sulfate had a less marked effect. Magnesium sulfate depressed bacteria more than molds. The chloride had essentially no depressing effect on molds, but, in absence of

added nitrate, considerably reduced the bacterial count. In most cases sodium nitrate greatly lessened the depressive action of the various salts.

Differentiation of the molds reveals that all salt treatments eliminated the Mucors; Penicillia, on the other hand, were little affected except by the carbonate, which was strongly depressive for this group. In contrast, the Aspergilli were markedly stimulated by sodium carbonate and sodium chloride; sodium sulfate had less influence, and magnesium sulfate virtually none.

The proportion of actinomyces was not greatly influenced, except for a material reduction in the presence of sodium chloride and sodium sulfate. *Azotobacter* was suppressed by sodium carbonate and by sodium chloride. The influence of the sulfate was undeterminable because of the rapidity with which molds overgrew the plaques. Magnesium sulfate was without apparent effect.

STUDIES ON VALE "ALKALI" SOIL

Microbial activities in a natural saline soil and in a partially reclaimed saline soil were studied in another series of experiments. These soils were obtained from the Vale Branch Experiment Station in eastern Oregon. The virgin soil is a moderately salinized heavy loam supporting semiarid desert vegetation, mainly sagebrush, greasewood, and salt grass. The reclaimed soil was taken from a plat treated with flour sulfur at the rate of 500 pounds an acre in 1932. This plat yielded 5,000 pounds of alfalfa hay in 1935, whereas check plats with corresponding irrigation yielded less than 100 pounds. Bulk samples were taken in April, 1936. Chemical properties of these samples are given in table 4.

Portions of each soil were treated with various materials as indicated in table 5, and water was added to give 60 per cent saturation. These samples were incubated in the respiration apparatus for 215 days at room temperature and sampled at 115 days and at 215 days for chemical analysis and microbial counts.

Effect of the various treatments on carbon dioxide production is shown in figures 2 and 3, the check being taken as zero. The results with the two soils were similar. Only with glucose did the reclaimed soil give a significant increase over the virgin soil. In general, the trend of carbon dioxide formation as affected by various treatments in the two soils is in the same order. In the virgin soil 1 ton of sulfur had a slight depressing effect, and 2 tons gave a slight increase; ammonium sulfate exerted essentially no influence. On the other hand, in the reclaimed soil 2 tons of sulfur caused depression, and 1 ton gave a slight increase; ammonium sulfate, again, had little effect.

The sulfur additions had a depressing influence on evolution of carbon dioxide from straw, the effect being most pronounced in the virgin soil. Nitrate was slightly depressive in the virgin soil, but gave reverse results in the reclaimed soil.

It is evident that carbon dioxide evolution carries additional significance for carbonate-bearing soils as compared with noncarbonate-bearing soils, since carbon dioxide liberated in decomposition of organic matter will react according to the following equation:

$$Na_2CO_3 + CO_2 + H_2O = 2\ NaHCO_3$$

Apparent carbon dioxide evolution is reduced in proportion to bicarbonate formed. Furthermore, carbonates may be decomposed by the various in-

TABLE 4

Chemical properties of virgin and reclaimed "alkali" soils from Vale experimental plats

(Surface 5 inches)

	VIRGIN	RECLAIMED
Moisture content of bulk sample (per cent)	17.6	30.3
Water capacity (per cent)	57.0	80.7
pH	9.82	8.75
Total nitrogen (per cent)	0.054	0.123
Organic matter (per cent)	1.31	1.84
Water-soluble salts:		
Total (p.p.m.)	3,150	1,310
CO_3 (p.p.m.)	1,019	0
HCO_3 (p.p.m.)	0	330
S as SO_4 (p.p.m.)	79	30
P as PO_4 (p.p.m.)	0.1	1.5
Cl (p.p.m.)	108	76
Na (p.p.m.)	1,200	969
Ca (p.p.m.)	76	125
Replaceable Bases:		
Exchange capacity (equiv. p.p.m.*)	242	129
Ca and Mg (equiv. p.p.m.)	25	98
Na (equiv. p.p.m.)	203	27
K (equiv. p.p.m.)	15	4
Ca as per cent of exchange capacity	10.2	75.2
Na as per cent of exchange capacity	83.8	21.0

* Equivalent p.p.m. = p.p.m. $\times \frac{1}{\text{equivalent weight.}}$

For discussion of this method of expression, see Bollen and Neidig, *Soil Sci.*, 24: 69–70 (1927).

organic and organic acids of microbial origin, thus giving rise to apparent increase in soil respiration. These various carbon transformations are well illustrated in table 9.

Table 5 indicates the change in mineral and biological carbon in virgin saline soil as affected by the treatments. The shift in mineral carbon shows a strong biological pressure effect; from 70 to 85 per cent of the total biological

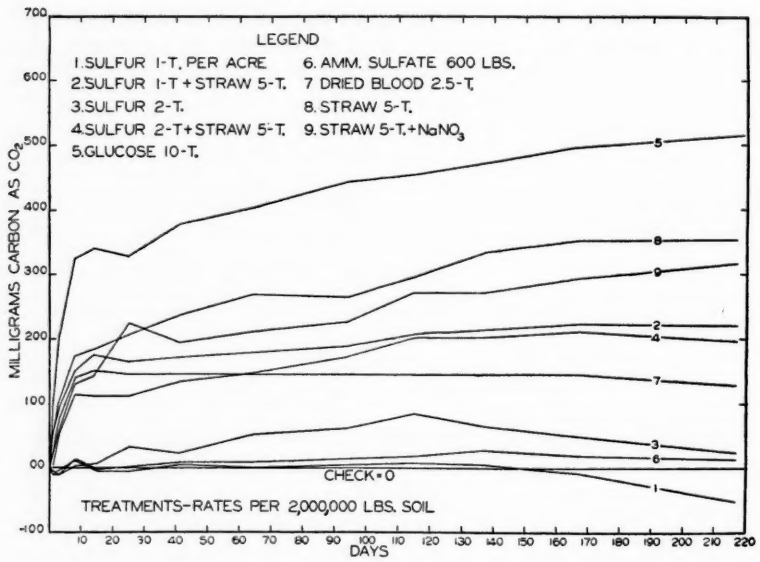


FIG. 2. EFFECT OF TREATMENT ON CARBON DIOXIDE EVOLVED FROM VIRGIN SALINE SOIL

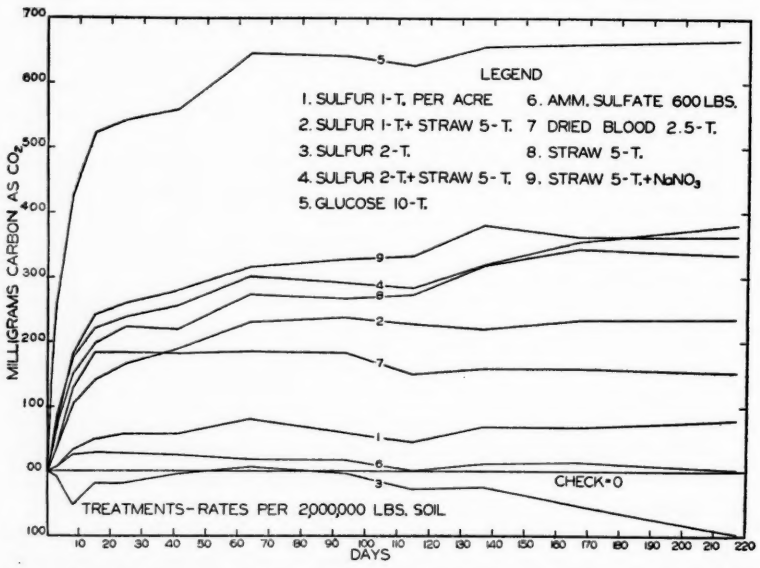


FIG. 3. EFFECT OF TREATMENT ON CARBON DIOXIDE EVOLVED FROM RECLAIMED SALINE SOIL

TABLE 5

Effect of treatments on change in mineral and biological carbon with time in virgin saline soil from Vale Experiment Station

TREATMENT	MINERAL CARBON						MINERAL CARBON EVOLVED AS CO_2*		TOTAL CARBON EVOLVED AS CO_2†		BIOLOGICAL CARBON EVOLVED AS CO_2‡	
	CO_3		HCO_3		Total							
	Incubation period, days											
	115	215	115	215	115	215	115	215	115	215	115	215
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
0. No treatment	120	100	14	80	134	180	70	24	675	930	605	906
1. Sulfur—1 T.§	24	0	123	67	147	67	57	137	682	878	625	741
2. Sulfur—1 T., straw—5 T.	36	0	121	61	157	61	47	143	881	1,150	834	1,007
3. Sulfur—2 T.	36	0	141	61	177	61	27	143	759	953	732	810
4. Sulfur—2 T., straw—5 T.	12	0	141	61	153	61	51	143	877	1,125	826	1,082
5. Glucose—1 per cent	108	60	61	92	169	152	35	52	1,130	1,444	1,095	1,392
6. $(NH_4)SO_4$—300 p.p.m.	60	60	122	88	182	148	22	56	692	943	670	887
7. Dried blood—2,500 p.p.m.	60	24	82	85	142	109	62	95	819	1,058	757	963
8. Straw—5 T.	60	90	102	82	162	172	42	32	970	1,281	928	1,249
9. Straw—5 T., $NaNO_3$—125 p.p.m.	60	102	100	98	160	200	44	4	947	1,246	903	1,242
Original sample	204		0		204							

* Total in original sample less total in treated portion.

† In respiration apparatus.

‡ By difference, total carbon minus mineral carbon.

§ Tons per 2,000,000 pounds soil.

carbon dioxide was produced during the first period. At the same time the carbonate-bicarbonate shift was most extensive. That carbon dioxide originating directly from microbial activity plays a considerable part in this shift is shown by results obtained with the untreated soil as well as with glucose and straw treatments. Additions which gave rise to an abundance of mineral acids produced, during the first half of the experiment, the greatest concentrations of bicarbonate; subsequently they also contributed to the most rapid bicarbonate decomposition. Although microbial activity decreased in the final period, carbon dioxide tension in the untreated soil was sufficient to increase bicarbonate from 14 to 80 p.p.m., more biological carbon dioxide being absorbed and converted to mineral combination. This absorption is accounted for only in part by the 20 p.p.m. decrease in normal carbonate. The remaining 46 p.p.m. must have been absorbed by bases from the exchange complex.

A greater conversion of biological carbon to bicarbonate occurred with the glucose treatment. Approximately 40 per cent more carbon dioxide was produced than in the untreated soil, and mineral absorption was much greater, especially during the first 115 days. This can be attributed to the ease with which glucose is fermented, resulting in an early rapid activity and high microbial pressure, which correspondingly increased carbon dioxide concentration in the soil. Action of organic acids is reflected by the materially lowered pH.

Carbonate was decreased approximately 85 per cent by all sulfur treatments in the first period. At the close of the experiment 70 per cent of the carbonate carbon had been evolved as carbon dioxide, and 30 per cent remained as bicarbonate. Although the larger application of sulfur was much more effective than the smaller application in lowering pH, the effect on carbonate was little different. Addition of straw with sulfur caused additional lowering of pH, probably because of buffering properties of organic decomposition products as well as of increased carbon dioxide production. Here again the carbonate relations were not appreciably altered. The course of sulfur oxidation is indicated both by increase in sulfate and by decrease in pH (table 9).

A comparison of straw alone with straw plus sulfur indicates that sulfur retarded decomposition in the virgin saline soil (fig. 2 and table 9). This may well be because the decomposing microorganisms require a relatively long period to become acclimated to a lower pH. There is similar evidence that acclimatization is incomplete in the reclaimed soil.

Table 7 shows that in the reclaimed soil the changes in mineral and biological carbon were similar to those of virgin soil. The only difference is the absence of normal carbonate in the reclaimed soil, mineral carbon being present entirely in the form of bicarbonate. Only metabolic acids other than carbonic can alter soil bicarbonates, and here again the microbial pressure was more effective during the first period than during the second period.

The application of various materials to the virgin soil resulted in an increase in the number of bacteria and molds, as indicated in table 6. Slight reduction occurred in the number of actinomyces. With few exceptions, the results show that the microorganisms tend to decrease in the second period. The contrary is true for *Azotobacter*, which showed no development in the first period and moderate to heavy development in the second period. In general, the various treatments were effective in increasing the numbers of microorganisms. Similar results obtained in the reclaimed soil (table 8), with the exception that in most cases moderate development of *Azotobacter* occurred

TABLE 6

Effect of treatments on microbial population in virgin saline soil from Vale Experiment Station

TREATMENT	BACTERIA*		ACTINOMYCES*		MOLDS†		AZOTOBACTER‡	
	Incubation period, days							
	115	211	115	211	115	215	115	211
0. No treatment	2.0	1.9	0.6	0.3	20	22	O	X
1. Sulfur—1 T.§	2.2	2.9	0.3	0.1	29	25	O	X
2. Sulfur—1 T., straw—5 T.	3.5	3.9	0.3	0.1	33	30	O	X
3. Sulfur—2 T.	5.5	5.7	0.2	0.1	33	29	O	X
4. Sulfur—2 T., straw—5 T.	7.0	6.8	0.3	0.1	24	20	O	X
5. Glucose—1 per cent	4.0	3.5	0.5	0.2	29	52	O	X
6. $(NH_4)_2SO_4$—300 p.p.m.	2.8	3.2	0.1	0.1	25	23	O	XX
7. Dried blood—2,500 p.p.m.	3.8	1.9	0.7	0.2	23	24	O	XX
8. Straw—5 T.	3.0	2.0	0.1	0.1	20	21	O	XX
9. Straw—5 T., $NaNO_3$—125 p.p.m.	2.7	1.9	0.2	0.1	23	20	O	X
Original sample	1.4		0.2		10		O	O

* Millions.

† Thousands.

‡ O = no development, X = moderate development, XX = heavy development.

§ Tons per 2,000,000 pounds soil.

in the second period as compared to the heavy development in the first period. This may have been due in part to the change in reaction.

The response of virgin and reclaimed soil to the various treatments is shown in table 9. Comparison of the data reveals that on the basis of biological carbon dioxide evolution the two soils respond essentially alike in all cases. All treatments had a marked effect in lowering the carbonate content of virgin samples; sulfur, the most effective treatment, completely eliminated normal carbonate by the close of the experiment. With the decrease in normal carbonate, bicarbonate increased and pH decreased. On the other hand, in the reclaimed soil, bicarbonate decreased, since no normal carbonate was present. The decrease was most pronounced with the sulfur treatments.

TABLE 7

Effect of treatments on change in mineral and biological carbon with time in reclaimed saline soil from Vale Experiment Station

TREATMENT	MINERAL CARBON						MINERAL CARBON EVOLVED AS CO_2*		TOTAL CARBON EVOLVED AS CO_2†		BIOLOGICAL CARBON EVOLVED AS CO_2‡	
	CO_3		HCO_3		Total							
	Incubation period, days											
	115	215	115	215	115	215	115	215	115	215	115	215
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
0. No treatment	0	0	41	40	41	40	25	26	644	901	619	875
1. Sulfur—1 T.§	0	0	41	31	41	31	25	35	690	983	665	948
2. Sulfur—1 T., straw—5 T.	0	0	41	24	41	24	25	42	872	1,138	847	1,096
3. Sulfur—2 T.	0	0	41	18	41	18	25	48	615	804	590	756
4. Sulfur—2 T., straw—5 T.	0	0	41	18	41	18	25	48	928	1,283	903	1,235
5. Glucose—1 per cent	0	0	53	24	53	24	13	42	1,271	1,568	1,258	1,526
6. $(NH_4)_2SO_4$—300 p.p.m.	0	0	41	33	41	33	25	33	644	902	619	869
7. Dried blood—2,500 p.p.m.	0	0	41	34	41	34	25	32	795	1,054	770	1,022
8. Straw—5 T.	0	0	53	31	53	31	13	35	918	1,236	905	1,201
9. Straw—5 T., $NaNO_3$—250 p.p.m.	0	0	53	31	53	31	13	35	977	1,264	964	1,229
Original sample	0		66		66							

* Total in original sample less total in treated portion.

† In respiration apparatus.

‡ By difference, total carbon minus mineral carbon.

§ Tons per 2,000,000 pounds soil.

There was a significant difference in the sulfur-oxidizing power of the soils. At the close of the experiments, 100 per cent of the sulfur added to the reclaimed soil was oxidized, and only 20 to 30 per cent of the sulfur was oxidized in the virgin soil. Sulfur oxidation in both soils was more extensive in the first period than in the second period. No change in sulfates occurred with other treatments, except where actual addition was made, as in the ammonium sulfate treatment. All treatments increased the amount of water-soluble phosphorus, sulfur being the most effective.

Nitrates were more abundant in the reclaimed soil than in the virgin soil under all treatments except dried blood, which was more completely nitrified

TABLE 8

Effect of treatments on microbial population in reclaimed saline soil from Vale Experiment Station

TREATMENT	BACTERIA*		ACTINOMYCES*		MOLDS†		AZOTOBACTER‡	
	Incubation period, days							
	115	215	115	215	115	215	115	215
0. No treatment	5.0	4.8	0.7	0.5	20	23	X	X
1. Sulfur—1 T.§	6.4	5.2	0.5	0.4	28	33	X	X
2. Sulfur—1 T., straw—5 T.	5.7	5.9	0.6	0.6	20	15	XX	X
3. Sulfur—2 T.	5.6	6.0	0.6	0.5	19	14	XX	X
4. Sulfur—2 T., straw—5 T.	5.6	5.5	0.5	0.3	24	27	XX	X
5. Glucose—1 per cent	5.4	5.6	0.6	0.7	22	26	X	X
6. $(NH_4)_2SO_4$—300 p.p.m.	5.4	4.6	0.6	0.4	28	26	X	X
7. Dried blood—2,500 p.p.m.	6.8	5.1	0.4	0.2	21	17	X	XX
8. Straw—5 T.	5.4	5.2	0.4	0.4	30	24	XX	XX
9. $NaNO_3$—125 p.p.m.	5.4	5.2	0.4	0.5	21	25	XX	XX
Original sample	4.6		0.5		9		X	

* Millions.

† Thousands.

‡ X = moderate development, XX = heavy development.

§ Tons per 2,000,000 pounds soil.

in the virgin soil. This better nitrification may have been due to the presence of normal carbonate, for it has been shown (2, 11) that sodium carbonate increases ammonification of dried blood. Where straw was added, nitrates decreased in the reclaimed but not in the virgin soil; there were no significant changes in carbon dioxide evolution to correlate with this. Ammonium sulfate was more completely nitrified in reclaimed soil. Because carbonates are a source of carbon dioxide in the presence of mineral acids, decrease in nitrification and sulfur oxidation, as well as lowered microbial activities in general, contributed to the decreased carbon dioxide evolution during the second period.

TABLE 9

Comparative response of virgin and reclaimed saline soils to sulfur and organic treatments

TREATMENT	INCUBATION PERIOD	CARBON EVOLVED AS CO_2				WATER-SOLUBLE SALTS										pH	
		Biological		Mineral		CO_3		HCO_3		Sulfur as SO_4		Phosphorus as PO_4		Nitrogen as NO_3			
		Virgin	Reclaimed	Virgin	Reclaimed	Virgin	Reclaimed	Virgin	Reclaimed	Virgin	Reclaimed	Virgin	Reclaimed	Virgin	Reclaimed	Virgin	Reclaimed
	days	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*		
Original sample	...	...	...	...	...	1,019	0	0	330	79	30	Tr.*	1.5	2	23	9.82	8.75
0. No treatment	115	605	619	70	25	600	0	68	205	83	28	Tr.	2.5	5	25	9.63	8.66
	215	906	875	24	26	501	0	398	198	84	29	2.0	3.1	6	30	9.76	8.63
1. Sulfur—1 T.†	115	625	665	57	25	120	0	616	205	275	1,110	Tr.	2.7	8	36	9.61	8.23
	215	741	948	137	35	0	0	336	153	398	1,250	7.5	15.7	9	45	9.26	7.60
2. Sulfur—1 T., straw—5 T.	115	834	847	47	25	180	0	603	205	301	1,110	Tr.	2.7	5	38	9.54	7.98
	215	1,007	1,096	143	42	0	0	305	122	435	1,250	7.5	15.7	7	45	8.96	7.41
3. Sulfur—2 T.	115	732	590	27	25	180	0	704	205	427	2,500	Tr.	2.9	4	33	9.54	7.88
	215	810	756	143	48	0	0	305	91	440	2,500	6.0	15.7	10	45	7.50	7.10
4. Sulfur—2 T., straw—5 T.	115	826	903	51	25	60	0	704	205	431	2,500	Tr.	2.7	0	23	8.68	7.87
	215	1,082	1,235	143	48	0	0	305	92	490	2,520	12.5	15.7	5	23	7.20	7.30
5. Glucose—1 per cent.	115	1,095	1,258	35	13	540	0	303	266	75	25	Tr.	2.7	7	45	8.96	8.57
	215	1,392	1,526	52	42	300	0	458	122	79	26	1.3	8.3	12	75	8.80	8.63
6. $(NH_4)_2SO_4$—300 p.p.m.	115	670	619	22	25	300	0	609	205	260	45	Tr.	2.7	30	75	9.55	8.59
	215	887	869	56	33	300	0	442	166	256	50	1.3	12.0	50	150	9.50	8.61

7. Dried blood—2,500 p.p.m..	115	757	770	62	25	300	0	409	205	83	26	Tr.	2.5	150	84	9.21	8.30
	215	963	1,022	95	32	120	0	427	169	84	25	1.3	8.5	194	156	9.20	8.28
8. Straw—5 T.....	115	928	905	42	13	300	0	510	266	83	26	Tr.	2.5	5	19	9.81	8.66
	215	1,249	1,201	32	35	450	0	412	153	84	25	1.3	4.5	8	Tr.	9.80	8.83
9. Straw—5 T., $NaNO_3$ —125 p.p.m.....	115	903	964	44	13	300	0	509	266	80	26	Tr.	2.5	10	20	9.82	8.69
	215	1,242	1,229	4	35	500	0	488	153	84	25	1.3	4.5	15	Tr.	9.81	8.74

* Tr. = less than 0.1 p.p.m.

† Tons per 2,000,000 pounds soil.

CONCLUSION

Notwithstanding an amount of salinity and alkalinity that is deleterious to agricultural plants, the virgin soil from the Vale Branch Experiment Station harbors an active and versatile microbial population, and the usual functions significant to soil fertility are neither lacking nor significantly restricted. Reclamation of moderately salinized soils of the Vale type can thus be regarded as desirable more from the standpoint of direct influence on crop plants than from benefits occurring indirectly through effects on soil microorganisms. The sulfur-oxidizing power of the reclaimed soil, however, is much greater than that of the virgin soil. This significant difference is to be expected from stimulation of the sulfur-oxidizing microflora by the sulfur added in reclamation. In a former investigation (9), it was found that sulfur-oxidizing efficiency in several widely different soil types had been increased by previous sulfuring.

Results obtained in the experiment with Willamette silty clay loam showed decidedly depressive effects from "alkali" salts, but these effects were obtained with concentrations much greater than those in the Vale soil.

SUMMARY

Carbon dioxide evolution in saline soils must be corrected for mineral carbon contributed or withdrawn by carbonates before it can serve as an index of organic matter mineralization. When so corrected, it is equal in importance to ammonification, nitrification, and other specific soil microbial activities in evaluating effects of salinity on functions significant to soil fertility.

Bio-organic decomposition was studied in an artificially salinized soil and in a natural solonetz under laboratory conditions by establishing a carbon balance which accounted for changes in carbonate and bicarbonate as well as for carbon dioxide evolution.

A slightly acid soil from the subhumid Willamette Valley region was treated with various "alkali" salts and incubated in a laboratory respiration apparatus for 164 days. Determination of carbon dioxide evolution and other soil microbial activities gave the following results:

All the salts depressed the rate of decomposition of the native soil organic matter as well as that of added straw.

Sodium chloride was most depressive; sodium sulfate, least depressive; and magnesium sulfate and sodium carbonate were intermediate in effect.

Nitrate accumulation was retarded in about the same order as carbon dioxide evolution.

Sulfur oxidation was not influenced by sodium chloride or sodium carbonate.

Water-soluble phosphate was indirectly influenced, being decreased as pH was increased.

Microbial function was similarly studied in a virgin "alkali" soil and in its reclaimed counterpart. The following results were obtained:

Reclamation has had little permanent influence on the native microflora and their activities in the Vale soil. Microbial response of the virgin soil to various additions was similar to that of the reclaimed soil. The latter, however, was superior in sulfur-oxidizing capacity.

The virgin soil nitrified dried blood and decomposed straw more effectively but was less effective in nitrifying ammonium sulfate and in decomposing glucose.

Normal carbonate was completely eliminated from the virgin "alkali" soil by sulfur oxidation; 70 per cent was eliminated as carbon dioxide, and 30 per cent was transformed to bicarbonate. Oxidation of native and added organic matter, as well as nitrification, produced similar but less extensive carbonate transformation. Bicarbonates in the reclaimed soil were reduced 40 to 70 per cent by microbial activity.

A microbial pressure effect is evident in the various transformations. This is greatest during the initial period of high activity stimulated by the establishment of optimum conditions, and is especially well revealed by carbonate-bicarbonate shifts.

REFERENCES

(1) BOLLEN, W. B. 1931 Improved hydrogen-electrode cell for determination of pH. *Indus. and Engin. Chem., Analyt. Ed.*, 3: 203–209.

(2) BROWN, P. E., AND HITCHCOCK, E. B. 1917 The effects of alkali salts on nitrification. *Soil Sci.* 4: 207–229.

(3) BROWN, P. E., AND JOHNSON, D. R. 1918 Effects of certain alkali salts on ammonification. Iowa Agr. Exp. Sta. Res. Bul. 44.

(4) FRED, E. B., AND WAKSMAN, S. A. 1922 A tentative outline of the plate method for determining the number of microorganisms in the soil. *Soil Sci.* 14: 27–28.

(5) GIBBS, W. M., BATCHELOR, H. W., AND MAGNUSON, H. P. 1925 The effects of alkali salts on bacteriological activities in soils: I, II, III. *Soil Sci.* 19: 343–356, 357–369, 371–379.

(6) GREAVES, J. E. 1916 The influence of salts on the bacterial activities of soil. *Soil Sci.* 2: 443–480.

(7) GREAVES, J. E. 1922 Influence of salts on bacterial activities of soil. *Bot. Gaz.* 73: 161–180.

(8) GREAVES, J. E., AND CARTER, E. G. 1921 The influence of moisture and soluble salts on the bacterial activities in the soil. *Soil Sci.* 13: 251–270.

(9) HALVERSEN, W. V., AND BOLLEN, W. B. 1923 Studies on sulfur oxidation in Oregon soils. *Soil Sci.* 16: 479–490.

(10) LIPMAN, C. B. 1909 Toxic and antagonistic effects of salts as related to ammonification by *Bacillus subtilis*. *Bot. Gaz.* 48: 105–125.

(11) LIPMAN, C. B. 1911 Toxic effects of "alkali salts" in soils on soil bacteria. *Centbl. Bakt.* (II) 36: 58–64.

(12) LIPMAN, C. B. 1913 Antagonism between anions as affecting ammonification in soils. *Centbl. Bakt.* (II) 36: 382–394.

(13) NEIDIG, R. E., AND MAGNUSON, H. P. 1924 Alkali studies: I. Tolerance of wheat for alkali in Idaho soil. *Soil Sci.* 18: 449–467.

(14) POTTER, R. S., AND SNYDER, R. S. 1916 Carbon and nitrogen changes in the soil variously treated with lime, ammonium sulfate and sodium nitrate. *Soil Sci.* 1: 76–94.

(15) RATHER, J. B. 1917 An accurate loss-on-ignition method for determination of organic matter in soils. Ark. Agr. Exp. Sta. Bul. 140.

(16) SCHREINER, O., AND FAILYER, G. H. 1906 Colorimetric, turbidity, and titration methods used in soil investigations. U. S. Dept. Agr. Bur. Soils Bul. 31.

(17) TRUOG, E., AND MEYER, A. H. 1929 Improvements in the Deniges colorimetric method for phosphorus and arsenic. *Indus. and Engin. Chem., Analyt. Ed.*, 1: 136–139.

FURTHER STUDIES OF BACTERIUM GLOBIFORME AND THE INCIDENCE OF THIS TYPE OF ORGANISM IN CANADIAN SOILS[1]

C. B. TAYLOR

Department of Agriculture, Ottawa, Canada

Received for publication April 21, 1938

The interest surrounding *Bacterium globiforme* has been less concerning its functions in the soil than its presence or absence in that medium, and the peculiar morphological change exhibited by the organism in turning from a definite rod-shaped cell to a coccus form. Conn (2) during a study of the so-called punctiform-colony-forming bacteria found that a large proportion of the organisms studied showed this change in morphology. It was felt that the presence of these coccus-forming rods in soil might account for the fact that direct microscopic examination of untreated soils revealed largely coccus-like organisms. In two later papers by the same author (13, 3) the ability of the type species, *Bact. globiforme* Conn, to grow in inoculated sterilized soils was tested. Two soils, Volusia silt loam and Hoosick course sandy loam, were found to be incapable of supporting growth of the organism. Both soils were highly acid, but it was found necessary to supply suitable nitrogen and carbon sources in addition to lime in order to produce growth of *Bact. globiforme.* The conclusion was drawn that although a soil supporting good growth of this microörganism does not necessarily possess a high crop-producing power, crops cannot thrive in a soil deficient in the substances required for the nutrition of the organism.

Further work was carried out by Conn and Darrow (5) on the same two soils. Growth tests were made on inoculated sterilized samples treated with organic and inorganic nitrogen sources and also with salts of the strong alkali metals. Results indicated that ammonium salts, nitrates, certain forms of organic nitrogen, hydroxides, sulphates, carbonates, and phosphates could promote growth of *Bact. globiforme.* The authors concluded that the two soils under investigation, although high in total nitrogen, lacked available nitrogen, and hence growth of *Bact. globiforme* could be obtained by addition of available nitrogen sources, or by conversion of the unavailable to available nitrogen by means of certain salts of alkali metals.

Conn (3) and Conn and Darrow (6) attempted to measure the utilization of glucose by *Bact. globiforme.* In preliminary experiments using a synthetic medium plus 1 per cent glucose it was found that 7 to 83 per cent of the sugar was utilized. Such a variation in consumption was attributed to the fact that as the

[1] Contribution No. 49 (Journal Series) from the Division of Bacteriology, Science Service, Department of Agriculture, Ottawa, Canada.

organism did not grow normally on agar, conditions for growth in liquid media would be still more abnormal. Later experiments showed that the highest percentage of glucose was utilized when the initial concentration was approximately 0.2 per cent. It was estimated that between 48 and 75 per cent of the carbohydrate was utilized and that 50 to 60 per cent of the carbon in that fraction was converted into CO_2.

A study of the utilization of nitrogen from 0.1 per cent ammonium phosphate showed that 60 to 90 per cent of the nitrogen furnished was consumed and that 70 to 80 per cent of that consumed was converted into cell substance.

The general conclusions reached by Conn and Darrow in their most recent work (6) on *Bact. globiforme*, which summarize most of their previous work, are worthy of note. The authors conclude that *Bact. globiforme* retains in the soil, nitrogen that has been converted by other microörganisms into a soluble form and which otherwise would have been removed by drainage or utilized by plants. Whether the action is harmful on account of competition of the organism for nitrate nitrogen, or whether it may be a beneficial action in prevention of loss of nitrogen by leaching is problematical. In view of the fact that *Bact. globiforme* is ordinarily more abundant in better soils, the beneficial action probably predominates. The organism is strictly aerobic and is well adapted to surface soil conditions, where, utilizing simple sources of nitrogen and very small quantities of carbohydrate, it converts inorganic material into cell substance which is readily converted into humus upon the death of the organism.

Taylor and Lochhead (11) determined numbers of *Bact. globiforme* in field plots and found the organism to be as prevalent in a soil of low fertility, cropped continuously for 25 years without application of fertilizer, as in plots of much greater crop-producing power to which farmyard or artificial manures had been applied. It was also observed that the metamorphosis from rod to coccus was not merely a shortening of the rod until the organism became spherical, but a swelling of the rod followed by a fragmentation, leaving ovoid bodies which became cocci.

More recently Topping (15), in an investigation of the predominant microorganisms in soils, found coccus-forming rods to be the most common type. These were divided into three groups:

Group 1. Gram-positive, motile bacteria which may produce branching variants.
Group 2a. Gram-positive, nonmotile, rod-shaped bacteria.
Group 2b. Gram-positive, nonmotile, mycelium-forming bacteria.

Relationship of *Bact. globiforme* Conn to group 2a is suggested by the author on the basis of the nonmotility of the organism and the fact that no branched forms had been noticed. Taylor and Lochhead (12), however, have since observed branched forms in cultures of *Bact. globiforme* Conn, and suggest that all three of Topping's groups contain strains of the *Bact. globiforme* group of organisms. Such a suggestion is strengthened by a comparison of photographs obtained by the respective authors.

In a previous paper (11) from this laboratory it was suggested that the inability to determine greater numbers of *Bact. globiforme* in soils of high crop-producing power than in a plot of low crop-producing power might be due to the fact that the latter soil had been rendered infertile by continuous cropping and hence was not strictly comparable with the naturally infertile soils with which Conn worked. To test this point it was decided to examine a large number of soils of different types and fertilities for the presence and abundance of *Bact. globiforme.*

MATERIAL AND METHODS

Through the coöperation of the Branch Farms and Stations of the Experimental Farms System, soil samples were obtained from widely scattered districts extending from as far east as Kentville, Nova Scotia, west to Saanichton, Vancouver Island, and as far north as Beaverlodge, Alberta, and Fort Good Hope, Northwest Territories.

The samples were typical of a large variety of different soils, from both the pedological and the agricultural aspects, and were representative of virgin, prairie, orchard, garden, pasture, and mixed cropped soils. Two or more samples, usually of different crop-producing power, were received from each district. Because of the time elapsing between the receipt of the soils and their sampling, it was thought best for comparative purposes to allow the samples to air dry at room temperature before examination.

The soils were plated on tap-water gelatin medium, and the plates were incubated 7 days at 18°C. A dilution of 100,000 was employed but in some instances proved to be excessive, hence replating at a lower dilution was necessary. Bacterial colonies appearing on the plates were counted, and from a sector of each plate 20 colonies were picked off and inoculated on nutrient agar slopes. After 5 days' incubation at 28°C. the subcultures were examined by means of preparations stained with gentian violet. Most of the organisms of the *Bact. globiforme* type were readily identified by their coccus form, but in many instances the organisms still appeared as rods and prolonged incubation was necessary for confirmation. With experience *Bact. globiforme* could be differentiated from true cocci; where any doubt existed as to a culture's being one or the other, subcultures were made and examined after 24 hours' incubation.

The pH values of the various soil samples were measured electrometrically by the quinhydrone electrode.

EXPERIMENTAL

Of 90 soils examined, *Bact. globiforme* was isolated from 89. Table 1 shows the approximate numbers of *Bact. globiforme* present in soils of different fertility and fertilizer treatment. It can be seen that, in general, numbers tend to be higher in the more fertile plots, although Lacombe soils are a notable exception. From the figures representing numbers as a percentage of the total

plate count, it would appear that higher numbers mean not necessarily a greater relative number of *Bact. globiforme* but a greater general population.

TABLE 1

Numbers of Bact. globiforme in soils of different fertility supporting rotational crops

LOCATION	TREATMENT*	FERTILITY	pH	BACT. GLOBIFORME	
				Count (100,000)	Per cent of plate count
Charlottetown, P. E. I.	A. + F.Y.M.	.....	5.6	5.5	43.0
	A.	.....	4.8	0.14	4.6
Lennoxville, Que.	A. + F.Y.M.	Medium	6.3	22.0	55.0
	A. + F.Y.M.	Low	5.3	13.0	51.0
Ste. Anne de la Pocatière, Que.	A. + F.Y.M.	High	6.6	35.0	45.0
	A. + F.Y.M.	.....	6.8	56.0	70.0
Cap Rouge, Que.	F.Y.M.	High	6.3	188.0	94.0
	A. + F.Y.M.	Low	4.5	4.2	37.0
Kapuskasing, Ont.	F.Y.M.	.....	7.7	129.0	86.0
	None	.....	7.8	95.0	95.0
Brandon, Man.	None	High	7.6	12.0	57.0
	None	(Wind-blown)	8.5	5.4	67.0
Indian Head, Sask.	None	High	8.3	51.0	100.0
	None	High	7.8	15.0	88.0
Scott, Sask.	None	.....	7.4	8.0	61.0
	None	.....	5.9	18.0	75.0
Swift Current, Sask.	None	Good	8.3	51.0	74.0
Lacombe, Alta.	None	High	6.5	10.0	50.0
	None	Low	6.2	54.0	88.0
Lethbridge, Alta.	A. + F.Y.M.	High	8.1	142.0	94.0
	A. + F.Y.M.	P_2O_5-deficient	8.0	85.0	85.0
Summerland, B. C.	F.Y.M.	Medium	8.5	36.0	70.0
	F.Y.M.	Lower	8.5	37.0	77.0
Fort Good Hope, N.W.T.	.....	.....		144.0	41.0

* A. = Artificial fertilizer; F.Y.M. = Farmyard manure.

In two soils, however, from Charlottetown, Prince Edward Island, and from Cap Rouge, Quebec, the pH values of which were lower than 5.0, it is evident

that *Bact. globiforme* is not only actually restricted but also represents a noticeably smaller percentage of the total plate count.

The effects of lime and fertilizer treatments are demonstrated in table 2. In one case, at Kentville, Nova Scotia, a series of plots cropped with a rotation of roots, grain, and clover since 1914 has been used to compare nitrate of soda with sulfate of ammonia, in each instance with and without lime. It can be seen that numbers of *Bact. globiforme* are increased enormously by the addition of lime and very slightly by the application of fertilizer. Not only were

TABLE 2

Effect of soil treatment on numbers of Bact. globiforme

ROTATION	FERTILIZER	AMENDMENT	pH	FERTILITY	BACT. GLOBIFORME	
					Count (100,000)	Per cent of plate count
Experimental Station, Kentville, Nova Scotia						
Roots, grain, clover	Nitrate of soda, superphosphate, basic slag, muriate of potash	None	5.6	.....	1.4	28
		Lime	6.5	.....	18	60
Roots, grain, clover	Sulfate of ammonia, superphosphate	None	5.5	.....	0.46	15
		Lime	6.3	.....	19	63
Roots, grain, clover	None	None	5.5	.....	1.4	45
		Lime	6.5	.....	12	60
Experimental Farm, Nappan, Nova Scotia						
Oats, hay	Nitrate of soda, basic slag	Lime	7.2	Medium	23	62
	None	Lime	6.1	Low	10	66
Swedes, oats, hay	Farmyard manure	Lime	6.5	High	33	70
	None	Lime	5.8	Low	9	64
Oats, hay	Basic slag	None	5.3	High	16	64
	None	None	5.0	Medium	10	55

the numbers increased by lime but also the proportion of the total plate count was materially increased, suggesting a group stimulation. In the other instance, figures are shown for fertilized and check plots from three rotational systems in operation since 1923 at Nappan, Nova Scotia. In all three cases numbers show a substantial increase with fertilizer treatment, most particularly so with farmyard manure. Increases are all proportional to the increase in the total plate count, and hence *Bact. globiforme* was not preferentially stimulated. The plot system did not permit deduction as to the effect of lime.

Table 3 includes a number of miscellaneous soils, such as garden, orchard, virgin, and Ontario tobacco soils. Apparently *Bact. globiforme* is as prevalent in virgin soils as in cultivated soils. The Swift Current sample is an exception: the *Bact. globiforme* count is very low, and a comparison with the other sample (table 1) taken from an adjoining cultivated field shows greater num-

TABLE 3

Numbers of Bact. globiforme in miscellaneous soils

LOCATION	SOIL	TREATMENT*	pH	BACT. GLOBIFORM	
				Count (100,000)	1 per cent of plate count
Agassiz, B.C.	Garden	F.Y.M.	6.7	29.0	50.0
	Orchard	None	8.3	10.0	50.0
Saanichton, B.C.	Garden	F.Y.M.	7.4	37.0	80.0
	Orchard	.....	6.0	12.0	60.0
Windermere, B.C.	Garden	F.Y.M.	8.3	53.0	80.0
	Pasture	Virgin	7.6	8.0	66.0
Beaverlodge, Alta.	Woodland	Virgin	7.0	72.0	92.0
	Woodland	Virgin	6.1	29.0	28.0
Melfort, Sask.	Prairie	Virgin	6.0	63.0	84.0
Swift Current, Sask.	Prairie	Virgin	7.7	0.97	54.0
Essex County, Ontario	Orchard	.....	6.0	6.0	55.0
	Tobacco	Artificials	6.2	17.8	72.0
	Tobacco	Artificials	6.2	0.9	51.0
	Tobacco	Artificials	6.3	2.2	58.0
	Oats	F.Y.M.	7.0	3.5	40.0
	Wheat	.....	7.3	40.0	88.0
	Wheat	F.Y.M.	6.0	18.0	80.0
Kent County, Ontario	Tobacco	Artificials	6.8	26.0	86.0
	Tobacco	Artificials	6.8	13.0	68.0
Parry Sound, Ontario	Oats	.....	6.0	0.0	0.0

* F.Y.M. = Farmyard manure.

bers in the cultivated soil. The Ontario soils had been collected and air dried several months before they were received at the laboratory, but relatively large numbers of *Bact. globiforme* were isolated from them in most instances. *Bact. globiforme* was not found in the Parry Sound sample, but this is believed to be due to inability to isolate the organism rather than to its absence. The gelatin plate count of this soil is only 200,000 per gram. Lowering the dilu-

tion sufficiently to allow the growth of 15 to 20 colonies results in the appearance of gelatin-liquefiers, which liquefy the medium before any slow growers can make an appearance.

DISCUSSION

The assertion of Conn (3) that general crops cannot thrive in a soil deficient in the substances required for the nutrition of *Bact. globiforme* may be correct, but the fact that from only one sample of soil, a soil that was air dried for several months, was *Bact. globiforme* not isolated, suggests that it is difficult to obtain such soils to test the theory. The results of this investigation show that, from a geographical standpoint, the organism is as abundant in a soil situated on the rim of the Arctic Circle as in soils on the Atlantic and Pacific coasts of southern Canada. It is present in garden, prairie, orchard, marsh, and mixed cropped soils, whether acid, neutral, or alkaline, fertile or infertile.

In general, counts of *Bact. globiforme* in soils do not provide any more information than can be obtained from a total plate count, but it would appear that in soils more acid than pH 5.0 this type of organism is severely repressed. This is interesting in view of the fact that the two soils in which Conn could initiate no growth of *Bact. globiforme* were as acid as pH 4.5. In point of fact, in an earlier paper (13) growth was reported when an unmeasured amount of $CaCO_3$ was added to the soil, but later (3) the statement was modified to the effect that the organism was virtually unable to grow. In the latter work the enormous dosage of 1 per cent $CaCO_3$ was employed. In view of the wide range of carbon and nitrogen sources that *Bact. globiforme* Conn is capable of utilizing, it is difficult to ascribe any reason, other than acidity or the presence of some toxic substance, for the failure of the organism to grow in soil.

MORPHOLOGY AND PHYSIOLOGY OF THE BACT. GLOBIFORME GROUP

In previous work (11) it was found that of the cultures isolated, many separate strains could be differentiated by their action on dextrose, by reduction or nonreduction of nitrates, and in some cases by pigmentation. Although all cultures showed the characteristic tendency to change from rod to coccus, the time taken to accomplish the metamorphosis and the cell size of the organism varied greatly between strains. The peculiar morphology of *Bact. globiforme* has lent itself to considerable speculation on the part of Conn and Darrow (6) and Topping (15) with regard to the systematic position of the organism. Although it is placed in Bergey's Manual (1) under the genus Achromobacter, Conn and Darrow do not accept this placing and state that the spherical form in soil suggests relationship to the cocci. In certain physiological aspects the organism resembles the Bacteriaceae. Topping considers that *Bact. globiforme* will eventually be included among the corynebacteria.

In order to understand more fully the relationship of the various strains of *Bact. globiforme*, the physiology of a large number of cultures isolated from widely different soils has been studied. All cultures were replated on beef-

peptone agar prior to the commencement of the experimental work. The morphology of a few strains has been examined in detail. Results have been compared with those of the type species of *Bact. globiforme* supplied by Dr. H. J. Conn.

Morphology in artificial media

The morphology of the organism is profoundly affected by time and temperature of incubation and by the nature of the substrate. On beef-peptone agar young cultures are consistently short rods which vary considerably in length, tend to be slightly curved, and tend to produce clumps. A few cocci and clavate cells are commonly present. At temperatures above 28°C. large distended rods with bulbous extremities, which break off into cocci, are prevalent. As the culture ages the rods turn into cocci by a process which is virtually a swelling and fragmentation of the rod. This swelling is much more obvious in some strains than in others, and with the Conn strain can be seen clearly only at incubation temperatures of 30°C. or more. It also seems probable that the rod can divide into two or more cocci, after the nature of *Rhizobium*. The time taken to attain the coccus state varies considerably. With some strains the metamorphosis is almost complete in 48 hours, and with others, 12 or more days may elapse before the appearance of an appreciable number of cocci.

In 3-week-old cultures, certain morphological differentiations can be made. These depend upon the size of the cocci and upon the presence or absence of appreciable numbers of rod forms. Such distinctions do not appear to be correlated with any other type characteristic.

On solid synthetic media, young cultures invariably show rod morphology. If such carbohydrates as dextrose, mannite, and xylose are incorporated, together with certain forms of nitrogen, the cocci peculiar to old cultures are absent, and larger oval cells in which a small deeply stained spherical form is embodied, make their appearance. Stained clumps of these cells strongly resemble frog spawn. When ammonium salts are used as the sole source of nitrogen, the organism becomes very pleomorphic, and long swollen and branched rods outnumber cocci, even in old cultures.

The morphology is more extreme in liquid media. Cultures in nutrient broth are present as slightly curved rods after 12 hours' incubation, but later become swollen or branched. After 3 days the disintegration of the branched forms is apparent, the bulbous sprouts and extremities of the cell breaking off as cocci. In old cultures the organism is present as rather large cocci, together with an assortment of pleomorphic rods.

Morphology in soil

In order that the morphology of the organism during its growth in soil might be compared with that on artificial media, portions of sterilized soil

were inoculated separately with four strains of *Bact. globiforme*, and glass microscope slides were inserted after the technic of Conn (4). Sets of jars were incubated at room temperature, at 28°C., and at lower outside temperatures. Slides were removed at intervals, stained, and examined. After 2 days' incubation, the organisms were rather sparse and were present as short rounded rods, with individuals having a banded appearance. The only difference between stains was that of a slight variation in cell size. After 8 days' incubation, the organisms were very dense in the soils kept at room temperature and at 28°C., and growth was abundant in the outside samples. The four strains of *Bact. globiforme* in all soils showed remarkable uniformity in morphology, all being present as slightly elongated cocci, only a small proportion of which could truly be said to be spherical enough to resemble staphylococci. In order to eliminate the effect of the glass-soil interface, large petri dishes were substituted for the jelly jars and the glass slides were omitted in a repetition of the work. Samples from these dishes, taken at daily intervals by means of a cork borer, were macerated with water, and stained smears were prepared. The results were similar to those of the previous experiment, and on no occasion could pleomorphic or branched rods be observed.

Nitrogen requirements

As a base for determining the availability of certain nitrogen sources, a synthetic semisolid medium of the following composition was prepared:

	gm.
K_2HPO_4	0.2
$MgSO_4$	0.05
NaCl	0.05
$CaCl_2$	0.025
Dextrose	10.0
Agar	3.0
Distilled water	1,000 ml.

To this basal medium were added separately, $NaNO_3$, $(NH_4)_2SO_4$, yeast extract, beef extract, peptone, cystine, and urea in amounts calculated to give 0.01 per cent nitrogen. From 106 cultures of *Bact. globiforme* stab inoculations were made into tubes of the above media. Such semisolid media proved unsuitable for good comparison of growth, and results were so variable that any attempt at classification on a basis of acid production from carbohydrate was futile. Most of the cultures used the semianaerobic nature of the nitrate medium to break down the nitrate completely. Dextrose was used as a source of energy, and an acidic reaction was produced in the medium. Growth and acid production in $(NH_4)_2SO_4$ and cystine media varied greatly. The complex organic sources of nitrogen—yeast extract, beef extract, and peptone—proved adequate for excellent growth, but the production of an acidic reaction was not consistent with the three compounds.

For a better comparison of growth, a solid medium of the same basal composition as that of the semisolid medium was prepared. With the exception of beef extract and peptone, all previous nitrogen sources together with the amino acids, alanine, asparagin, glycine, and leucine, were employed. Slopes of the media were inoculated with the various cultures and examined after 3 and 8 days' incubation. Results showed that the cultures of *Bact. globiforme* could be divided into two types, those utilizing nitrate nitrogen, and those unable to do so. The former produced luxuriant growth, and the latter, slight or no growth on nitrate medium. Both types grew equally well with yeast extract as a source of nitrogen. Only 17 cultures of the 106 examined, including *Bact. globiforme* Conn, belonged to type I. The relative availability of the nitrogen sources was as follows:

Type I: Yeast extract, Asparagin, Glycine, $NaNO_3$ > Leucine, Urea, Alanine > $(NH_4)_2SO_4$, Cystine (slight growth)

Type II: Yeast extract > Leucine, Glycine, Asparagin (poor growth) > $(NH_4)_2SO_4$, $NaNO_3$, Alanine, Cystine, Urea (trace or no growth)

Production of acid was confined chiefly to those media in which $(NH_4)_2SO_4$ and leucine were incorporated; this was probably due to the formation of sulfuric and leucic acids respectively. Sufficient acid may have been produced from $(NH_4)_2SO_4$ to restrict the growth of the organsims on this medium, which would be necessary to conform with the work of Koser and Rettger (8), who showed that ammonia and amino nitrogen (including urea) are qualitatively equivalent as sources of nitrogen for bacteria. Such an explanation would seem justified, as organisms of type I grew well in Conn's ammonium phosphate-dextrose medium, whereas organisms of type II grew poorly or not at all.

Relative numbers of the two types, together with some cultural characteristics, are shown in figure 1.

Carbon sources

The availability of different carbon compounds for organisms of type I was readily determined by the use of a synthetic medium with $NaNO_3$ as the nitrogen source. Preference was as follows:

Dextrose, Mannite > Inulin, Glycerol, Raffinose, Xylose > Lactate, Citrate, Acetate, Starch, Dextrin

Usually the strains made very little growth where lactate, citrate, acetate, dextrin, or starch was the carbon source, but two strains of those tested made good growth. An acidic reaction was not produced from dextrose or glycerol, and the inconsistent and changeable reactions produced from other carbon sources only emphasized the futility of any attempt to classify the organisms on the basis of acid production.

The inability of organisms of type II to utilize inorganic or simple organic nitrogen sources necessitated employment of a more complex form, yeast extract, and hence an additional carbon source was present. Results from a few compounds tested indicated that dextrose and mannite were much more available sources of carbon and energy than were raffinose, xylose, or inulin.

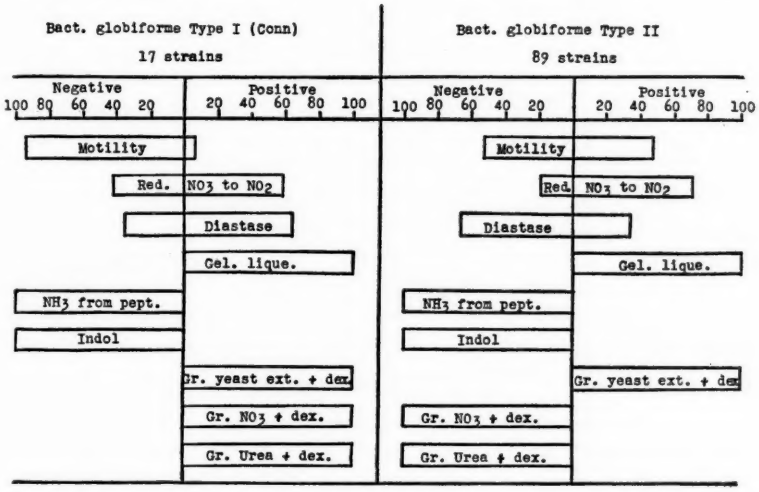


FIG. 1. CORRELATION OF CHARACTERS OF BACT. GLOBIFORME, TYPES I AND II

EFFECT OF ENVIRONMENT

Plants

Sterilized seeds of alfalfa, red clover, and timothy were sown in 8-inch by 1-inch test tubes containing a sterile nitrogen-free medium described by Thornton (14). Upon the germination of the seeds, separate tubes were inoculated with four strains of *Bact. globiforme*. No growth of the organisms was visible for the first 10 days, after which, with the aid of a magnifying glass, small bacterial colonies could be observed surrounding the rootlets of the seedlings. The colonies increased in size with time until they became visible to the naked eye and spread a considerable distance along the roots. As the plant matured, colonies became less apparent and finally disappeared. The plants showed no beneficial or harmful effects, in either root or foilage growth,

from this bacterial invasion. The attraction of *Bact. globiforme* to the roots of plants is of some interest in view of the recent work by Starkey (10), who reported that coccoid bacteria were among the most numerous types encountered in a microscopic examination of the rhizosphere.

Temperature

Strains of *Bact. globiforme*, as shown previously, can survive in soils exposed to subzero temperatures. By exposing sterilized soils which had been reinoculated with several strains of *Bact. globiforme* to various temperatures, it was ascertained that the organisms are not so tolerant of heat as of cold. Only one culture survived an exposure to 45°C. for 24 hours, and it was killed at 50°C. These types of organisms are evidently among those eliminated by partial sterilization of the soil. The ranges of growth temperatures for various strains of *Bact. globiforme* vary considerably, and though 25°C. approximates the optimum growth temperature on nutrient agar, most strains produce some growth at 33°C. The Conn strain is the only one found to grow actively at 37°C.

Acidity

As a test of the tolerance of *Bact. globiforme* to acidity, 28 cultures were inoculated on slopes of synthetic media of various pH values. A comparison of growth was made after 5 days' incubation at 28°C., and results showed that, of the 28 cultures tested, 14 showed no growth at pH 4.0, 6 made some growth, and 8 grew well. Growth of the 14 cultures was severely repressed at pH 5.0, but all cultures grew equally well at pH 6.0, 7.0, and 8.0.

GENERAL CONSIDERATIONS

Outstanding characteristics of members of the *Bact. globiforme* group of organisms are their lack of uniformity in behavior, both morphological and physiological, and the strong pleomorphism influenced by the substrate. Although the change from a rod to a coccus or coccoid form invariably takes place regardless of the substrate employed, the time taken to complete such a transformation varies considerably from strain to strain. The pleomorphic rods, branched forms, and yeast-like cells which may be observed in artificial media have not been encountered in the soil and the question arises: Upon which of these forms should a morphological classification be made? Topping (15) asserts that the production of branched forms suggests relationship to the Proactinomycetacae. Conn and Darrow (6), on the other hand, consider that the existence of *Bact. globiforme* in the soil in a coccoid form would indicate relationship to the cocci. The similarity of *Bact. globiforme* and *Rhizobium* in their utilization of dextrose, a complete oxidation resulting in cell substance and CO_2 only, led Conn and Darrow (6) to speculate whether *Bact. globiforme* might be related to the Bacteriaceae. Such a basis of relationship is not strengthened by the fact that Merrill (9), studying the breakdown of dextrose by the Mycobacteriaceae, was likewise unable to determine the presence of any product other than CO_2. The systematic position and

relationship of coccus-forming rods, including those reported by Topping (15) and the Mycoplana group of Gray and Thornton (7), might well be investigated.

The 106 strains of *Bact. globiforme* studied were so diverse in such biochemical activities as the production of nitrite from nitrate, formation of diastase, and production of acidic reactions in various carbohydrate media that no two cultures proved to be identical. The ability or inability to utilize nitrate nitrogen aerobically is believed to be a better basis of separation of organisms belonging to the rod-coccus type than is the presence or absence of motility. *Bact. globiforme* Conn is representative of the former type, which is much less prevalent in the soils examined than is the latter type. Neither group has been found to be indicative of any particular type of soil.

SUMMARY

Of 90 soils examined, selected from widely separated points of Canada, 89 showed the presence of *Bact. globiforme*. Numbers of this organism show no relationship to fertility, but may be greater in fertile soils than in infertile soils, since fertile soils usually support a higher general bacterial population.

In virgin, prairie, garden, orchard, woodland, and mixed cropped soils, provided these soils are more alkaline than pH 5.0, *Bact. globiforme* is present in large numbers. Where application of fertilizer results in an increase in numbers of *Bact. globiforme*, the increase is not a preferential stimulation of this group but is proportional to the increase in total plate count. In soils more acid than pH 5.0 the organism is severely repressed; the application of lime to such soils increases numbers of *Bact. globiforme* in preference to other types.

The morphology of several strains of *Bact. globiforme* has been compared during growth on artificial media and in the soil. Certain pleomorphic forms observed in laboratory media have not been found to occur in soil.

The carbon and nitrogen requirements of 106 cultures of *Bact. globiforme* have been tested. Results have shown that cultures of this organism may be divided into two definite types on the basis of their nutrition, namely:

Type I. Capable of utilizing complex nitrogenous compounds, nitrate, certain amino acids, and urea as sources of nitrogen.

Type II. Capable of utilizing only complex nitrogenous compounds as sources of nitrogen.

Type II was found to be far more common in soils examined than was type I. Organisms belonging to both groups have been found to vary in respect to their biochemical reactions. When present in the soil, all show a marked resistance to low temperatures and to air drying, but are destroyed by exposure of the soils to a temperature of 45°C. for 24 hours. On artificial media more acid than pH 4.5 the majority of strains are unable to grow.

Strains of *Bact. globiforme* tested proved capable of proliferating around the roots of clover, alfalfa, and timothy seedlings growing in a sterile nitrogen-free nutrient medium.

REFERENCES

(1) BERGEY, D. H. 1934 Manual of determinative bacteriology, ed. 4. The Williams & Wilkins Co., Baltimore.

(2) CONN, H. J. 1925 Soil flora studies. VI. The punctiform-colony-forming bacteria in soil. N. Y. Agr. Exp. Sta. Tech. Bul. 115.

(3) CONN, H. J. 1928 A type of bacteria abundant in productive soils, but apparently lacking in certain soils of low productivity. N. Y. Agr. Exp. Sta. Tech. Bul. 138.

(4) CONN, H. J. 1932 A microscopic study of certain changes in the microflora of soils. N. Y. Agr. Exp. Sta. Tech. Bul. 204.

(5) CONN, H. J., AND DARROW, M. A. 1930 Influence of various non-nitrogenous compounds on the growth of certain bacteria in soils of low productivity. N. Y. Agr. Exp. Sta. Tech. Bul. 172.

(6) CONN, H. J., AND DARROW, M. A. 1935 Characteristics of certain bacteria belonging to the autochthonous microflora of soil. *Soil Sci.* 39: 95–110.

(7) GRAY, P. H. H., AND THORNTON, H. G. 1928 Soil bacteria that decompose certain aromatic compounds. *Centbl. Bakt.*, (II) 73: 74–96.

(8) KOSER, S. A., AND RETTGER, L. F. 1919 Studies on bacterial nutrition. The utilization of nitrogenous compounds of definite chemical composition. *Jour. Infect. Diseases* 24: 301–321.

(9) MERRILL, M. H. 1930 Carbohydrate metabolism of organisms of the genus Mycobacterium. *Jour. Bact.* 20: 235–285.

(10) STARKEY, R. L. 1938 Some influences of the development of higher plants upon the micro-organisms in the soil: VI. Microscopic examination of the rhizosphere. *Soil Sci.* 45: 207–227.

(11) TAYLOR, C. B., AND LOCHHEAD, A. G. 1937 A study of *Bacterium globiforme* Conn in soils differing in fertility. *Canad. Jour. Res.* C, 15: 340–347.

(12) TAYLOR, C. B., AND LOCHHEAD, A. G. 1938 Qualitative studies of soil micro-organisms. II. A survey of the bacterial flora of soils differing in fertility. *Canad. Jour. Res.* C, 16: 162–173.

(13) THATCHER, L. M., AND CONN, H. J. 1927 The bacterial flora of four soils compared by the direct microscopic method. N. Y. Agr. Exp. Sta. Tech. Bul. 129.

(14) THORNTON, H. G. 1930 The early development of the root nodule of lucerne (*Medicago sativa*, L.). *Ann. Bot.* 44: 385–392.

(15) TOPPING, L. E. 1937 The predominant micro-organisms in soils. I. Description and classification of the organisms. *Centbl. Bakt.* (II) 97: 289–303.

PLATE 1

CULTURES OF *Bacterium globiforme* CONN

FIG. 1. *Bacterium globiforme* Conn, 12-hour culture on beef-peptone agar at 28°C., gentian violet stain, showing rod stage. × 1040.

FIG. 2. *Bacterium globiforme* Conn, 48-hour culture on beef-peptone agar at 37°C., gentian violet stain, showing bulbous rods. × 1040.

FIG. 3. *Bacterium globiforme* Conn, 48-hour cultures in nutrient broth at 28°C., gentian violet stain, showing branched forms. × 1040.

FIG. 4. *Bacterium globiforme* Conn, 48-hour cultures in nutrient broth at 28°C., gentian violet stain, showing branched forms. × 1040.

FIG. 5. *Bacterium globiforme* Conn, 2-week culture on synthetic medium containing dextrose and cystine, gentian violet stain. × 1040.

FIG. 6. *Bacterium globiforme* Conn, 2-week cultures on synthetic medium containing dextrose and $NaNO_3$, gentian violet stain, showing oval yeast-like cells. × 1040.

FIG. 7. *Bacterium globiforme* Conn, 2-week cultures on synthetic medium containing xylose and $NaNO_3$, gentian violet stain, showing deeply staining bodies. × 1040.

FIG. 1

FIG. 2

FIG. 3

FIG. 4

FIG. 7

FIG. 5

FIG. 6

OXIDATION-REDUCTION POTENTIALS IN SOILS

AMAR NATH PURI AND ANAND SARUP

Irrigation Research Institute, Lahore, India

Received for publication July 19, 1937

The oxidation-reduction potentials (E_h) of soils have been studied by several workers, and the literature on the subject has been summarized by Brown (1). The important findings in the fields of bacteriology, biology, and biochemistry, made possible by the use of oxidation-reduction potentials, have resulted, however, in little fundamental work on soils. The practical importance of such measurements on soils have been visualized but not realized in actual practice. Even the theoretical background is vague, and no one has demonstrated in a convincing manner the nature of the oxidant and the reductant responsible for this phenomenon, though the presence of such substances in soils could not be denied. The present paper deals chiefly with this aspect of the problem, namely, the true cause of the so-called oxidation-reduction potentials in soils.

THEORETICAL CONSIDERATIONS

The fundamental equation involved in oxidation-reduction potentials is

$$E_h = E_o - \frac{RT}{nF} ln \frac{[Red]}{[Ox]}$$

where E_h is the potential in volts, referred to the normal hydrogen electrode as zero, of the system under consideration; E_o is a constant characteristic of the system and equal to E_h when the ratio $\frac{[Red]}{[Ox]}$ is unity; [Red] and [Ox] represent the concentrations of reductant and oxidant respectively; and R, T, n and F have their customary significance (2, p. 367).

The equation shows E_h to be dependent on the ratio of oxidant to reductant, as well as on temperature and on the particular system under investigation. It is not generally realized that the ratio of oxidant to reductant is the most important factor in the thermodynamic relation. Wherever a definite potential difference is to be established at the electrode, there must be in the system two species, one of which is the direct or indirect reduction product of the other, and the ratio of their concentrations or activities must be of finite magnitude. Neglect of this principle is not unusual in soil literature. It must be emphasized that a system which does not reversibly maintain a finite ratio of oxidant and reductant, leaves the electrode functioning in a manner that prevents the interpretation of any results obtained. Some potential will

always be developed when a clean platinum electrode is dipped into a soil solution; but unless the thermodynamic equations can be satisfied by the introduction of known components, the results will have no fundamental value and may in fact be meaningless.

The universal presence of iron in all soil systems might reasonably be assumed and all potentials interpreted in terms of the system $Fe^{+++} + E \rightleftarrows Fe^{++}$, but we cannot avoid the possibility of the participation of other systems when we are dealing with a substance like soil. The results in this case can be valuable and interpretable only if the characteristic data for one particular system can be obtained under conditions which preclude interference by another system. The relation between pH and E_h is practically linear for a system in which the ratio $\frac{[Red]}{[Ox]}$ is constant. The relation is complex when both this ratio and pH change simultaneously, and to obtain a complete picture a figure in three dimensions is necessary. Fortunately in soils the only possible system is $Fe^{+++}.F^{++}$, which gives a constant potential for a given ratio, irrespective of the pH value. On the other hand, the range of this system is limited because at high pH values iron is precipitated. We are, therefore, confronted with a dilemma in the case of soils. The thermodynamic relation might be satisfied by the fundamental equation for the system $Fe^{+++}.Fe^{++}$, but the quantity of iron that can remain in solution above pH 5.0 becomes so small that the system would cease to function as such.

EXPERIMENTAL

In order to determine the quantity of ferrous and ferric ions that can be brought into solution from the soil, a black cotton soil of high base-exchange capacity was shaken with different buffer solutions, and ferrous iron and ferric iron were determined in the filtrate. The results are given in table 1. It will be seen that virtually no iron is brought into solution above pH 5.5, and virtually no Fe^{+++} above pH 5.0. The Fe^{+++}. Fe^{++} system, therefore, is operative only up to pH 5.0. As this pH range is scarcely ever found in natural soils, we are forced to the conclusion that this system plays no part in determining the oxidation-reduction potentials in natural soils. It is doubtful, therefore, whether any measurements of E_h on agricultural soils with the object of correlating soil behavior in the field are likely to be fruitful.

It is of interest, however, to see whether as a result of eliminating extraneous influences, the exchange complex in soils would exhibit oxidation-reduction potentials which could be interpreted in terms of some known system. It is obviously impossible to study the influence of varying $\frac{[Red]}{[Ox]}$ ratios at constant pH in soils because we do not know even the nature of these ratios. There is, however, a possibility that under ordinary circumstances this ratio for a particular soil might be constant, in which case a study of the relation between pH

and E_h would be helpful in throwing light on the nature of this phenomenon in soils.

TABLE 1

Ferric and ferrous iron, in grams per 100 gm. of soil, brought into solution at different pH values

pH	FERRIC IRON	FERROUS IRON	TOTAL IRON
	gm.	*gm.*	*gm.*
0.65	0.1333	0.1167	0.2500
0.91	0.1046	0.1069	0.2115
1.42	0.0602	0.0939	0.1541
1.85	0.0256	0.0898	0.1154
2.32	0.0208	0.0753	0.0961
2.64	0.0132	0.0584	0.0716
3.09	0.0033	0.0341	0.0374
3.61	0.0064	0.0255	0.0319
3.95	0.0054	0.0234	0.0288
4.39	0.0040	0.0204	0.0244
4.76	0.0026	0.0170	0.0196
5.20	0.000067	0.0100	0.0101
5.50	0.000000	.....	.....

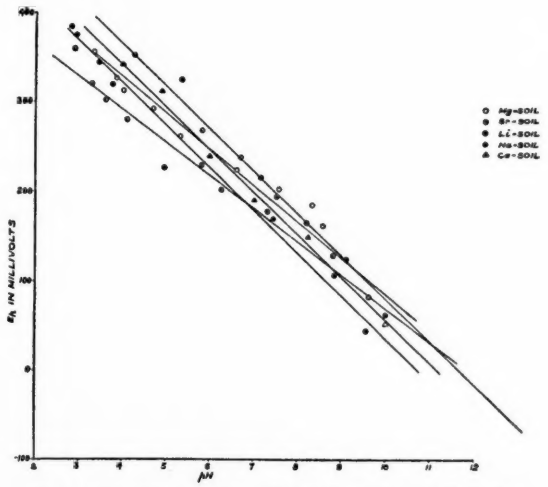


FIG. 1. RELATION BETWEEN pH AND E_h OF SINGLE-BASE SOILS

Soils used for this study were treated with 0.05 *N* HCl to remove all the exchangeable bases and carbonates. They were then shaken with increasing amounts of alkalies, and the relation between pH and E_h was determined. This relationship was found to be linear for all soils, irrespective of the nature

of the alkali used for titration. Some typical curves showing this relation are given in figure 1. The slopes of such straight lines for a number of soils are given in table 2. They indicate millivolts of change in potential for every unit change in pH. These values were obtained by the method of least squares, though a less accurate value could be obtained graphically by fitting a straight

TABLE 2

Change in potential in millivolts (Slope) for unit change in pH

SOIL NUMBER	SLOPE	SOIL NUMBER	SLOPE	SOIL NUMBER	SLOPE
M Series		*P.C. Series*			
1	59.5	2	68.5	48	39.3
2	58.7	4	37.4	49	46.5
3	66.1	6	69.7	50	67.5
4	44.4	7	56.44	53	65.3
5	43.0	9	54.8	54	56.5
6	63.1	10	48.9	56	31.18
7	56.8	11	41.8	57	60.96
8	51.8	12	68.5	58	35.72
9	67.5	14	51.03		
10	60.3	17	55.1		
11	53.8	20	53.5		
12	46.2	21	47.8		
13	52.2	26	73.1		
14	40.6	27	26.8		
15	46.0	28	28.8		
16	57.2	29	39.09		
17	63.0	30	65.0		
18	64.5	31	55.4		
19	56.5	33	76.3		
20	56.5	39	64.5		
21	54.0	44	57.52		
22	64.9	45	68.73		
23	50.1	47	27.69		

Gelatin	65.61
Humic acid	52.36
Iron silicate	65.02
Aluminum silicate	32.63

line to the pH $-$ E_h values. It will be seen that the mean value of the slope for all the soils is of the order of 53.4 millivolts.

It is remarkable that Michaelis (3), some years ago, pointed out the analogy between the micellas of a colloid acid (acidoid) and a metallic platinum-hydrogen-electrode, and ventured the conjecture that the potential of such an electrode might depend on the hydrogen-ion concentration according to the same logarithmic rule as the potential of a metallic electrode, that is, a varia-

tion of one pH unit should correspond to a change of 58 millivolts. Michaelis was unable to substantiate this conjecture experimentally while working with mastic particles, and he admitted that the analogy did not hold. We find that his original assumption was fundamentally correct and that his choice of mastic as a typical acidoid was unfortunate. We find that not only is the relation substantially correct for soils, but that other substances, like gelatin, humic acid, and aluminum and iron silicates, all of which furnish ionic micellas and behave as acidoids, give a straight line relation between pH and E_h, as

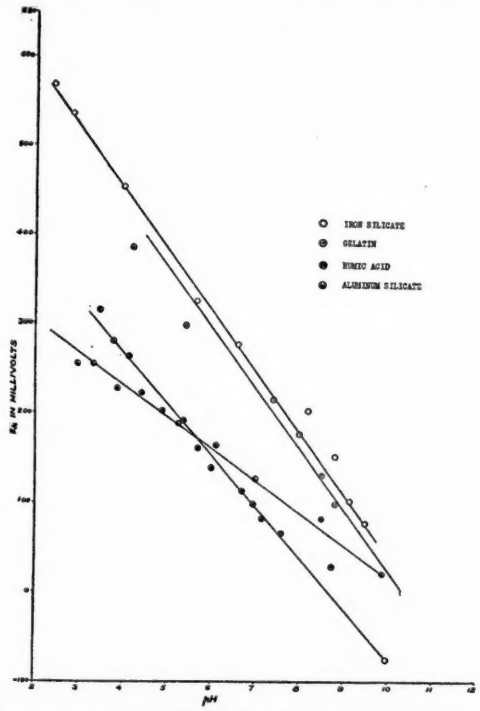


FIG. 2. RELATION BETWEEN pH AND E_h OF VARIOUS COLLOIDAL SUBSTANCES

shown in figure 2. In a metallic-hydrogen electrode, according to Nernst, the electrode has a tendency to emit hydrogen ions into the solution. On the other hand, the hydrogen ions of the aqueous solution have a tendency to settle on the metallic surface. One or the other tendency prevails, according to the concentration of hydrogen ions in the solution. Exactly the same thing happens in completely unsaturated soil, in which the tendency for the hydrogen ions to be liberated and to diffuse into the aqueous solution is the greater. This tendency is gradually diminished as the acidoid is neutralized with a base.

It will be seen that the thermodynamic relation does not include any factor relating to the quantity of the acidoid, and therefore the pH — E_h relation should be independent of the concentration of the soil suspension. A novel application of this principle is that a small quantity of an acidoid could be used for stabilizing the pH — E_h relation of an ordinary buffer. This will be clear from figure 3, which gives the pH — E_h relation in a series of buffer solutions before and after the addition of small amounts of clay suspension and finely powdered zeolite (sodium-aluminum silicate). The quantities of clay and zeolite added were so small that the pH value of the buffer was not affected, yet the potential was stabilised. It will be recalled that only a small quantity of quinhydrone is required to stabilize the pH — E_h relation in a

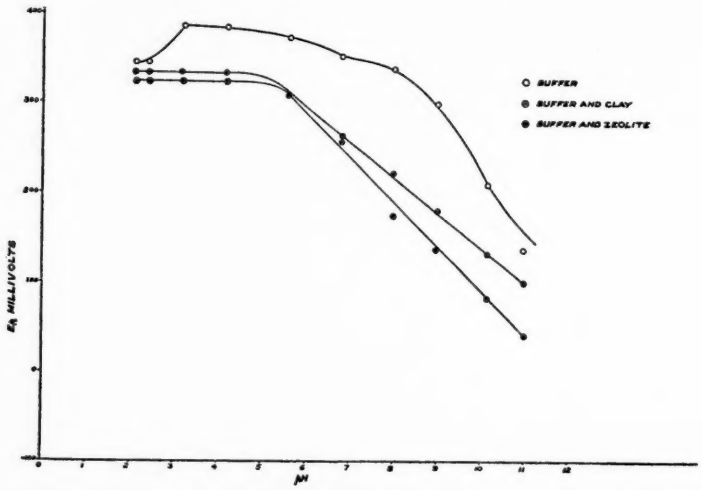


FIG. 3. STABILIZATION OF pH — E_h RELATIONSHIP BY CLAY AND ARTIFICIAL ZEOLITE IN BUFFER SOLUTION

solution, and this principle forms the basis of pH determinations by the quinhydrone electrode. Similarly, a small quantity of a colloidal solution that behaves as an acidoid could be used for measuring the pH value of solutions. The possibility of developing such an acidoid electrode for pH measurements should be explored by other investigators. Such an electrode would cover the alkaline range up to pH 10 and would thus be superior to the quinhydrone electrode.

With such perfect relationship between pH and E_h, it is hardly to be expected that the measurement of oxidation-reduction potentials in soils could lead to results of any greater value than those that could be obtained by pH measurements.

SUMMARY

The so-called oxidation-reduction potential in soils is shown to be a fundamental property of all colloidal substances which furnish ionic micellas and behave as acidoids. The relation between pH and E_h in soils is so perfect that the measurement of pH should suffice for all practical purposes.

REFERENCES

(1) Brown, L. A. 1934 Oxidation-reduction potentials in soils: I. Principles and electrometric determinations. *Soil Sci.* 37: 65.

(2) Clark, W. M. 1928 The Determination of Hydrogen Ions, ed. 3, Williams & Wilkins, Baltimore.

(3) Michaelis, L. 1925 General principles of the effects of ions in colloids. *Colloid Symposium Monog.* 2: 1.

A MECHANICAL DEVICE FOR DETERMINING THE PERMANENT WILTING POINT OF SOILS BY MEANS OF THE COHESION METHOD[1]

GEORGE JOHN BOUYOUCOS

Michigan Agricultural Experiment Station

Received for publication May 5, 1938

The cohesion method[2] has been offered as a rapid and practical means of determining the permanent wilting point of soils. The principle of the method is that at or above the wilting point the soil moisture film is sufficiently thick to cause the soil particles and granules to cohere when lightly pressed together, but below the wilting point the moisture film becomes too thin and discontinuous and is held with such great attractive forces that no such cohesion occurs.

The original procedure of the method consists of placing a small amount of moist soil on a base and lightly pressing the soil with a spatula, which is then quickly lifted. If the soil moisture is at or above the wilting point, the soil particles will stick to one another and to the spatula and will be lifted as a pressed soil mass or bar; and if the moisture is below the wilting point, the soil particles will not cohere, nor will they adhere to the spatula.

The accuracy of the method depends to a considerable degree on the amount of pressure that is applied to the soil by the spatula. Since the pressure applied to the spatula is by the hand, it is evident that the personal element enters into the technic and the experimental results are bound to vary with different operators. An automatic device for pressing the soil uniformly and thus eliminating the personal element in the technic thus becomes essential.

It is the purpose of this paper to present such a mechanical device, which increases the convenience, accuracy, and reliability of the cohesion method for determining the wilting point of soils. This mechanical device can also be used under field conditions to ascertain whether the field moisture is at, above, or below the wilting point.

DESCRIPTION OF MECHANICAL DEVICE

The mechanical device developed is shown in plate 1.[3] It consists of two rollers, both of which have handles. One roller, made of aluminum, is 2

[1] Authorized for publication as journal article 324, n.s., of the Michigan Agricultural Experiment Station.

[2] Bouyoucos, G. J. 1938 A rapid method for determining the permanent wilting point and for indicating under field conditions the relation of soil moisture thereto. *Soil Sci.* 45: 47–55.

[3] The base and roller shown in plate 1 are manufactured by the Metal and Wood Products Co., Bloomfield Hills, Michigan.

inches long, 1 inch in diameter, and weighs about 73 gm. This roller is to be used for all soils except the heavy, sticky clays. The other roller, made of brass, is 1–5/8 inches long, 1–5/8 inches in diameter, and weighs about 500 gm. This second roller is to be used only for the heavy, sticky clays. These rollers are allowed to rest on the base, and by means of the handle, they are slowly and gently rolled over the soil. Since their weight is constant and rests on the base, the pressure they exert on the soil when they are rolled over it tends to be uniform.

The following pieces of apparatus are required for the operation of the method with the special rollers:

Two special rollers.
A base painted with asphalt paint, waxed, and polished so that the soil will not stick to it.
A medium-sized 2-mm.-mesh sieve.
A small bottle of pure methyl alcohol. The neck of the bottle should be wide enough to permit the rollers to be dipped into the alcohol.
A pint Mason jar with cover.
An oilcloth about 12 inches square.
A medium-sized soil pan.
A 10-cc. pipette.
A small 3-inch-blade spatula.

For determining whether the soil moisture under field conditions is at, above, or below the wilting point, the procedure consists of taking a sample of the soil to be examined, screening it rapidly on the oilcloth, and putting it into the Mason jar. By means of the spatula, about 3 gm. of the soil is placed on the base and spread thickly (1/16 inch) and evenly without being compacted. The spreading or leveling is done by the edge of the spatula. The roller suitable to the soil type is dipped into the alcohol, shaken vigorously several times to throw off any excess alcohol, brought to rest on the base near the soil, and then, by means of the handle, it is slowly and gently rolled over the soil and lifted. If the soil moisture is at or above the wilting point, the soil particles will stick to one another and to the roller and will be lifted as a pressed or rolled soil mass (pl. 1); and if the soil moisture is below the wilting point, the soil particles will not cohere, nor will they adhere to the roller.

For determining the wilting point of soils in the laboratory by this improved cohesion method, the procedure is as follows: Approximately 50 gm. of an air-dry soil is weighed out and placed in a medium-sized soil pan. A definite amount of water is added to the soil, which is then mixed by hand, screened, mixed again, put into the Mason jar, and covered to prevent loss of moisture. The soil is then subjected to the sticking tests already described. If the test is negative, that is, if the soil particles do not stick to one another and to the roller, more water is added and mixed with the soil. The procedure of adding more water and mixing the soil thoroughly is continued until a positive test is obtained. At this point the total moisture is determined in the usual manner, and this moisture content represents the wilting point of the soil.

The number of additions of water to the soil and the quantity of water to be added at the outset depend on the type of soil. If the soil, for instance, is sand, the wilting point of which may be about 2 per cent, only about 0.5 cc. of water is added at first to 50 gm. of soil. If the soil is a clay loam, which has a wilting point of about 20 per cent, 7 or 8 cc. of water is added at first, and 0.5 to 1 cc. after each test. In this manner the procedure can be carried out fairly rapidly.

COMMENTS

The improved cohesion method, using the automatic device instead of the spatula, has been compared with the direct method and the dilatometer method of determining the permanent wilting point of soils. The results obtained are essentially the same as those presented in the first report, namely, that the cohesion method tends to give virtually the same wilting point determinations as do the other two methods. This is generally true in all the types of soil except in the extremely sticky clays as typified by Houston clay, McKenzie clay, and some of the clays of the lower soil horizons. In such extremely sticky clays the method is not highly accurate because of the great difficulty of sieving these clays and thereby bringing about a thorough and even distribution of the moisture on all their particles. For such extremely sticky clays, the dilatometer is the most accurate method of determining the wilting point. On the other hand, the method works fairly satisfactorily on clays which contain a considerable amount of silt or organic matter and which are not extremely sticky, such as Yolo clay, Capay adobe, and Davidson clay. With patience and care, the method can be made to work satisfactorily even with the extremely sticky clays.

Soils that have an appreciable content of salts tend to give a lower wilting point with the cohesion method than they do with either the direct method or the dilatometer method. This is probably because the cohesion method measures the wilting point that is due mainly to the physical attractive forces of the soil for the water, whereas the other two methods measure, in addition, the wilting point that is due to the salts. The differences, however, are small unless the soil contains large amounts of soluble salts.

When the wilting point is being approached the soil begins to stick and to lift partially and irregularly. This is not the point at which to stop. Additional amounts of water should be added until the soil sticks and lifts in a clear-cut manner without any dust being left behind (pl. 1) and until the same result can be obtained repeatedly. When the soil reaches the true wilting point it ceases to be in a powder form, and its original color changes because of the thicker moisture film that is formed around the soil particles. After the soil reaches the wilting point, additional water does not increase perceptibly its sticking and lifting phenomena as measured by this method. Very little water need be added, therefore, after the soil shows signs of sticking and lifting.

Silts tend to allow a premature cohesion and lifting to take place. When this occurs, it is noted that the base is not clean but is smeared with dust. The true wilting point is reached when the lifting of the soil leaves no dust behind and the base is absolutely clean. This is a very important point to watch, especially with silts.

All soils should be passed through a 2-mm.-mesh sieve and thoroughly mixed so that the moisture will be well distributed throughout the mass, which must be in a loose and granular condition. When the method is used in the field the sieving and mixing procedure should be done as rapidly as possible in order to avoid evaporation.

After the 50 gm. of soil is mixed with a definite amount of water and put into the Mason jar, any portion of the soil that is taken out for testing is returned to the jar when the test is completed. This is done by scraping the soil off the roller and off the base into the Mason jar. In this way one knows how much water to add for an approximate increase in water content. When the wilting point is approached the soil should be left in the jar for a few minutes after an addition of water, to allow the water to wet and to penetrate the soil particles thoroughly.

The object of dipping the roller in alcohol is to form a thin film on the roller and thus to facilitate the adhesion and lifting of the soil mass. This alcohol film is analogous to the water film around the soil particle. Any liquid can be used, but alcohol is very convenient for forming a thin film. When taken out of the alcohol, the roller should be shaken vigorously several times in order to throw off any excess alcohol and leave only a very thin film.

When the soil, especially clay, is being wetted by the addition of water, it indicates a slightly lower wilting point than it does when it is being dried by evaporation. This is due partly to hysteresis and partly to the difference in the moisture content between the outer and the inner surface of the soil granules in the two procedures.

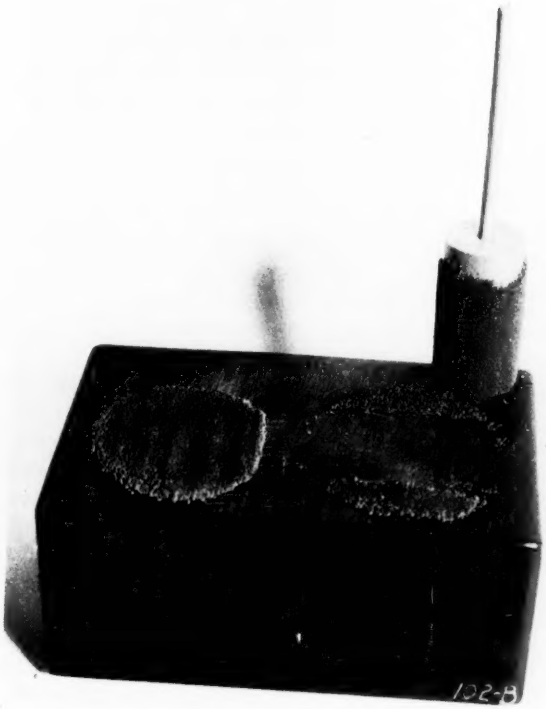
An attempt should be made always to take about the same volume of soil and to spread it evenly about 1/16 inch thick.

The 500-gm. roller should be used only for the heavy, sticky clays and not for the light soils. Because of their granular condition after being sieved, the true clays need to be pressed considerably harder than do the light soils.

PLATE 1

BASE, ROLLER, PILE OF SOIL BEFORE BEING PRESSED AND AFTER BEING PRESSED, AND PRESSED SOIL LIFTED BY ROLLER

PLATE 1



ON THE FORMATION OF STRUCTURE IN SOIL: VI. METHOD OF MICROSCOPIC INVESTIGATION OF SOIL STRUCTURE IN REFLECTED LIGHT

D. I. SIDERI

Agricultural Institute, Voronezh, U. S. S. R.

Received for publication March 24, 1938

The chief thesis of the theory of structure formation, developed by the author, is that the components of soil—sand, clay, humus, oxides—form particular combinations, which are mechanical mixtures, subject to definite laws.

The distribution of these components and their mutual orientation were studied in mixed formations isolated from soil (9) and in models representing mixtures of soil components (10). It proved possible to study, by optical, X-ray, and physicochemical methods, the properties of these mixed formations, both isolated from soil and prepared artificially. Studies by all these methods consistently showed that the union of the individual components of soil is due primarily to molecular forces, that is, to cohesion which is similar to attraction. Interaction between individual particles depends on their reciprocal orientation. Evidently, the particles can have no more favorable orientation than a parallel disposition along the line uniting two particles. The energy of interaction in this case is attractive force, the so-called "orientation effect" by which Keesom (2) explains the attraction of Van der Waals.

For the solution of problems bearing on the formation of soil structures, the introduction of the conception of definitely associated groups may prove more useful than the conception of the rôle of the absorbing complex and the absorbed bases in the soil. The existence, in soils, of structures similar to those described by the author for various mixtures of the individual components of soil (9, 10), may be presumed. It appeared, therefore, that further progress of the author's theory of structure formation might consist in the development of a method of investigation by which evidence of this might be obtained. After some preliminary work, it was found possible to use for this purpose a method of studying structure in polished sections prepared by methods similar to those used in mineragraphy (5, 6, 7, 14, 16) and metallography.

THE METHOD OF PREPARING POLISHED SECTIONS

Compact hard soil samples, free from large grains of sand or of other mineral elements, can be readily polished without the use of any cementing substance. The sample is polished first on a lathe of the type which is used for polishing minerals and which is fitted with a horizontal iron disk rotated by an electric

motor, fine corundum powder (the so-called "one minute" No. 15) moistened with water being used as the abrasive. Polishing on the lathe is continued just long enough to produce a flat surface. The next operation consists in polishing by hand on a glass plate, finer powders ("one minute" Nos. 120 and 240) being used this time. The sample is then polished on a small lathe, the disk of which is covered with chamois. With these methods, polished samples of different clay rocks, such as the Crimean "kil," Zhuravlin galloisite, and "volkonskonite" (clay containing chromium oxides), as well as samples of dried sols and gels of soil colloids and clay suspensions, were easily prepared. All these reflected light from their surfaces so well that they were readily photographed with the aid of the opaque-illuminator described by Van der Veen (14).

The polishing of clay rocks or soils containing sand or other mineral fragments presents much greater difficulties than does that of solid clay. The chief difficulty is in the heterogeneity of the material. In comparison with the clay minerals, the grains of quartz or of other minerals are much harder and more resistant to treatment. As a result, hollows and uneven places, hindering the further polishing of the sample, are formed. Colophony or fir resin was used, therefore, as cementing material for such samples. The material was dissolved in xylene (1:1), and the samples were immersed in this solution, which was slightly heated to facilitate permeation. As is known from earlier works (8) and from my recent observations, permeation of soil with an "inert" liquid does not disturb or distort the soil structure. It must be noted that when a soil sample is immersed in a viscous solution of colophony or fir resin the penetration of the cementing substance is limited to the surface layer of the sample. Polishing, therefore, must be done very cautiously, otherwise the cemented layer may be worn off entirely by prolonged polishing on the lathe and the cementing will have to be repeated. A suitable method of polishing is that elaborated for friable rocks of sedimentary origin (3, 4).

With some experience, the polishing of cemented samples, even those of small size, becomes easy. For samples of the shape of small grains, the method usually applied to small grains of minerals is recommended. The grains are cemented, melted into a plastic mass, and then polished in the manner already described. The method of polishing cemented samples by hand is similar to that of polishing uncemented samples, and the burnishing is done, likewise, with chamois. With most samples, we were able to obtain polished sections with good reflecting surfaces, a necessary condition for the microscopic study of structure.

LAMINAR STRUCTURE OF CLAYS

In earlier works of the author (9), it was established that in drying clay gels, a laminar structure becomes clearly visible, by reason of a great number of dark lines lying parallel to the length of the structural formation. These

lines, which are visible in natural light as well as between the Nicol prisms, show that the particles of clay are arranged in equidistant layers.

In studying polished sections of various clays in reflected light, a laminar structure having the same characteristic features as those of colloidal clay is observed. Figure 1, plate 5, a polished clay section ("volkonskonite"), illustrates this resemblance; it clearly shows how the particles of clay unite into equidistant layers. A similar laminar structure was manifested not only by samples of the Crimean clay "kil" and of the Zhuravlin galloisite, but also by polished sections prepared from dried gels of clay suspensions.

Figure 2, plate 5, shows the structure of a gel lamina obtained, by slow drying, from a Na-sol isolated from a covered clay (clay "Toida"). The clay sol was evaporated in a Petri dish. The lamina of desiccated gel was not polished. Its structure shows some features resembling those of the structure of a clay rock taken during the construction of the Moscow underground railway (the so-called "red clay," belonging to the Carbon period). This clay contains much fine quartz sand, which cannot be separated from clay colloids. The structure of this clay is shown in plate 5, figure 3, which clearly indicates *that there is no sharp limit between the grains of sand and the clay substance* (pl. 5, fig. 2). This indicates that sand-clay mixtures are subjected to the law of *mutual orientation with formation of a laminar system* possessing anisotropic properties (10).

STRUCTURE OF SOIL

Because of its practical importance, the structure of the superficial horizons of soil enriched with humus is of particular interest. Earlier researches of the author concerning the interaction of the organic matter and the mineral fractions of soil have shown (10) that the organic matter may be considered as a "metasomatic" substance, which transforms the parent rock and endues it with new properties.

The characteristic feature of this interaction is that no chemical reactions, probably, take place. The component parts of the mixed organic matter-clay formation occupy several states of relative equilibria (10). One of these corresponds to the growth of the clay crystal at the expense of "foreign" material, that is, organic matter, and represents a kind of pseudomorphosis. Another state of equilibrium corresponds to the isotropic condition of humus, which is characterized by a lesser stability of the bond between humus and clay. In each of these cases, we are dealing with a nonhomogeneous formation, consisting of two homogeneous substances, the particles of which are mutually oriented in different ways.

The maximum strength of the bond between the organic matter of soil and clay should be observed at the point at which the anisotropic particles of clay act on the isotropic humus in such a way that the particles of humus become anisotropic. In this case the mixed clay-humus formation must have a structure similar to that of pure clay (10).

The method of polished sections supplies fresh corroboration of this viewpoint. The following experiment was arranged. Flat laminas of desiccated Na-sol isolated from clay "Toida" were cemented with the following sorts of humates: a humate isolated from peat (Na-humate, which I have used in all my experiments); a water extract of decomposed manure, freed, by dialysis, of the admixture of salts and then concentrated by evaporation at a low temperature; and a humate obtained from brown coal (supplied by Z. J. Lein). The cementing, in every case, was done with a drop of humate placed between the laminas of clay. The set was almost instantaneous. After the desiccation of the laminas of clay, a cross section, including the laminas and the cementing substance between them, was polished. Microscopic examination of this section showed that the cementing layer of humate in close contact with the laminas of clay acquires a laminar structure indistinguishable in its characteristics from the laminar structure of clay. The rest of the humus does not form a laminar structure. The different kinds of humates employed for cementing the laminas of clay behaved similarly, as was shown by microscopic examinations of cross sections, and their structure was not different from that shown in figure 4, plate 5. Thus, this photograph may serve as an illustration of the orientation of humus particles in relation to clay and as a confirmation of the theory that clay is the substance which directs crystallization of humus.

On the basis of these observations, the conclusion may be drawn, that the formation of a watertight structure in soil is determined by the orientation most favorable to the forces of attraction, that is, parallel disposition of the particles of the mixed clay-humus formation. The checking of this conclusion on durable structure is of considerable interest and is achievable only by the technic employed for these structure studies.

Different samples of structural soils, the stability of which was checked by means of aggregate analysis, that is, by resistance to the destructive action of water, were chosen for observation. Among these were several samples of the solid granular structure of chernozems, of the solid structure of weak solonetz soils (supplied by V. V. Bernikov), and of watertight aggregates of a podzol soil from under clover (supplied by F. J. Heltzer from an experimental field of the Timiriazev Agricultural Academy).

The polished sections prepared from these water-resistant structural forms showed under the microscope *all the peculiarities of structure which characterize the presence of a firm bond* between the individual components of soil. This is illustrated in plate 5, which shows a polished section of the granular structure of a weakly leached chernozem from the Irtysh steppe (fig. 5), a columnar solonetz from West Siberia (fig. 6), and a watertight aggregate of podzol soil from under clover (fig. 7). In every one of these structures the particles of the mixed clay-humus formation are arranged in parallel layers.

The orientation of the particles of a mixed formation is so characteristic a feature for judging the firmness of the bond that it can be utilized for various

purposes, for example, for the comparative study of the structures of different horizons of a soil.

Utilizing the analysis of the structure of the plowed and underlying layers of a soil from the Kuznetzk Experiment Station, made by Vladychensky (14) according to the method of Tyulin (13), I made a comparative study of the structure of these horizons. Figure 8, plate 5, shows a polished section of soil from the plowed layer; figure 9, a polished section from the underlying layer. It is apparent from these photographs that we are dealing with soil formations related by their genesis but differing in that the bond between the particles of soil from the plowed layer is considerably weakened in comparison with the underlying layer. This observation corresponds with the results of the analysis made by Vladychensky (14).

It is necessary to dwell on one more observation of some significance in the clarifying of our theories regarding the influence of absorbed sodium on structure. It is well known that chernozem soil, saturated with sodium, becomes very unstable in regard to water, though it acquires considerable mechanical resistance. It is also known that chernozem soil, saturated with sodium under pressure, becomes watertight. My observations on Kamennaya steppe chernozem, saturated with sodium and then slightly washed with water to eliminate the salt, showed that the soil retains its macrostructure, provided it is left standing undisturbed, not only in water but also in an alkaline solution; even after the elmination of a considerable part of the organic and mineral colloids, the macroaggregates showed considerable stability and did not disintegrate when stirred with a glass rod (12).

Microscopic study of its structure explains the behavior of this soil; the structure is represented in plate 5, figure 10. Patches of disoriented black particles are visible amid particles oriented into parallel rows. As is shown by analysis, these disoriented areas do not consist of humus alone, but include mineral colloids as well (12). The presence of nonoriented particles is the cause of the loss by the soil, when elutriated gently in water or in a solution of NaOH, of those particles which are not associated into groups, that is, the disoriented part. On the other hand, those particles of the mixed formation which are oriented in rows remain, thus determining the shape and stability of the macroaggregates, in spite of the fact that these macroaggregates have remained for a long time in water or in a solution of alkali (12). The laminar structure of these aggregates is confirmed also by observations on thin polished sections of the same Kamennaya steppe soil between Nicol prisms (pl. 5, fig. 11). As is to be expected, the laminar formation, consisting of the sand-clay-humus mixture, is doubly refracting. There is no sharp limit between the individual components of the mixture. Sand, clay, and humus appear as a homogeneous mass with a uniform structure. Only a few coarse grains of sand escape from the general grouping.

When the amount of humus in the clay-humus mixture exceeds the number

of centers of crystallization, excess humus assumes the state of equilibrium corresponding to its isotropic condition. When such a mixture is desiccated it separates into two different parts: one, the anisotropic, has a high viscosity and is a pseudomorphous formation of humus on clay; the other, the isotropic, is less viscous and consists of humus. The disintegration of such a mixture is due to the surface tension of isotropic humus, which is nearly equal to that of water and which causes the humus to accumulate on the periphery of the more viscous anisotropic formation (11). The photographs of a humus-rich columnar solonetz from Volchia River (pl. 5, fig. 12 and 13) may serve as evidence that this phenomenon is encountered in soil. They represent polished vertical (fig. 12) and horizontal (fig. 13) sections of a columnar solonetz aggregate. It is evident that the structures of the sections are alike. Their characteristic feature is the structure of microaggregates about 0.3–0.5 mm. in size. The structure of these microaggregates corresponds exactly to that of a disintegrated clay-humus mixture containing excess organic matter (10, 11). The "amorphous" envelopes of isotropic humus deposited on the periphery of the anisotropic formation, which occupies the central position, are clearly visible in figures 12 and 13, plate 5. The anisotropic properties of this formation can be clearly distinguished in plate 5, figure 14, taken from a thin polished section of solonetz between crossed Nicol prisms.

STUDY OF STRUCTURAL CHANGES

As an example of a structureless soil, we may take a serozem from the Ak-Kawak Experiment Station (Central Asia). According to Heltzer (1), the watertight microaggregates (0.25–1 mm.) in this soil constitute only about 1.5 per cent of the total. Examination of polished sections prepared from the soil showed an almost complete absence of oriented groups of particles (pl. 6, fig. 1).

As the experiments of Heltzer have shown, the introduction of an insignificant amount of the autolytic products of the fungus *Aspergillus niger* completely transforms the structureless soil into a structural soil containing 99.6 per cent of stable macroaggregates (1). These structural changes were followed in several samples of the Ak-Kawak serozem moistened with the products of autolysis of *Aspergillus niger*. Several polished sections were prepared, all of which showed the same picture of alterations (pl. 6, fig. 2). Envelopes of another substance with a pronounced tendency to an oriented arrangement of particles, forming at the surfaces of delimitation of the separate disoriented groups of particles, are clearly visible in this figure. It is evident that the appearance of oriented envelopes is the cause of the abrupt rise in the stability of the serozem structure. It is noteworthy that this observation on the oriented arrangement of particles is in accordance with the previously established ability of autolytic products to form laminar systems manifesting strong double refraction.

Besides serozem, samples of chernozem from the Plavsky Machine and

Tractor Station were also investigated. This soil, according to Heltzer, has a very unstable structure. The samples, moistened with autolytic products of *Aspergillus niger* so as to produce thick and thin envelopes of this substance, were studied with regard to the structural changes taking place. Polished sections were prepared from every sample. Figure 3, plate 6, shows a sample of this soil untreated by the products of autolysis. The presence of disoriented groups of particles indicates that the soil is almost structureless. Quite a different picture is revealed by the photographs made from the samples of Plavsky chernozem covered with a thin layer (pl. 6, fig. 4) or a thick layer (pl. 6, fig. 5) of autolytic products. These figures show that the introduction of a small amount of the substance (fig. 4) changes completely the grouping of particles, endueing it with features characteristic of a stable granular structure (compare plate 5, figure 5). This confirms again the general rule concerning the high cementing capacity of thin layers of adhesive substances. The adhesive material is here limited to a very small volume in direct contact with large particles. The structure of the thick envelopes of autolytic products is clearly visible in figure 5, plate 6. The central part of the figure, representing the area of weakly oriented or quite unoriented groups, is surrounded by a thick layer of autolytic products having a characteristic structure.

An illustration of the influence of fertilizers and of agrotechnical methods on the structural changes in soil, is presented in field experiments with sugar beets in a two-field crop rotation of peas and sugar beets, arranged at the Suma Agricultural Experiment Station. The scheme of the experiments from which the soil was taken was as follows: Experiment 1. Control, no fertilizers. Experiment 3. Every second year, 60 kgm. P_2O_5 as superphosphate + 90 kgm. K_2O (K_2SO_4). Experiment 4. Every second year, 52 tons of manure. Experiment 18/20. Under sugar beets in the pea-sugar beet crop rotation, 52 tons of manure (12). The structure of soil samples from these experiments was studied in detail by means of aggregate analysis and by a special method of colloid-chemical separation (12).

The results of these analyses are in accordance with those of the microscopic studies of structure in polished sections. Figures 6–9, plate 6, represent samples of soil taken from experiments 1, 3, 4, and 18/20. Figure 6 (control experiment) shows fewer oriented particles than do the others. The characteristic feature of the soil structure of the control experiment is that the individual quartz grains are scattered irregularly, thus showing the lack of structure. The structure of soil from experiment 4 (fig. 7) bears a general resemblance to that of the control experiment, but differs from the latter in having a higher content of oriented praticles in laminar formations, easily discernible in different parts of the photograph. The composition of aggregates and the results of the colloid-chemical analysis show that the soils of experiments 1 and 4 are, indeed, very similar, differing to only a slight extent in their macroaggregate (0.5 mm.) content and in the loosely held fraction of the colloids (12). The structure of soil from experiment 3, with mineral ferti-

lizers (fig. 8), contains more oriented particle groups than that of soil from experiment 4, but quartz grains are still easily discernible here. This structure change corresponds to the analytical results: the quantity of macroaggregates (0.25–0.5 mm.) increases to 17.5 per cent as compared with 9.05 per cent in the control. The structure of soil from experiment 18/20, treated with manure in a pea-sugar beet crop rotation (fig. 9), differs from the others in that here the quartz grains are almost indistinguishable and represent an integral part of the laminar formations that are typical of the entire polished section. There is here no loosely held colloidal fraction (disoriented particles). The quantity of aggregates (0.25–0.5 mm.) is almost equal to that of the soil from experiment 3.

On the basis of this study of the structure of the soils of experimental plots treated with manure, it is impossible to draw the conclusion that a supply of "amorphous," that is, loosely bound, humus has formed in the soils under the influence of manure. The soil is "unsaturated" with humus, as is evidenced by the results of the colloid-chemical analysis (12).

SUMMARY

The observations described furnish grounds for the belief that the method of studying structure in reflected light by means of polished sections can be applied to the solution of a number of practical questions bearing on agrotechnical methods and the use of fertilizers. The method proved very sensitive in observations on structure changes and can, therefore, be used as a control and for purposes of comparison with other methods of soil structure analysis. The significance of this method is not limited to its applicability to practical problems. Microscopic study of structure may be utilized for the explanation of questions bearing on the genesis of soils, and particularly those on soil morphology. In problems of morphogenesis and in establishing the relation between the form of structural varieties and the inherent properties of soil, this method may be of considerable service, and may be of help in obtaining knowledge of the properties of soil.

REFERENCES

(1) HELTZER, F. J. 1937 The rôle of organic substance in the formation of soil structure. *Chemization Socialist Agr.* 8: 53–63 [Russian].

(2) KEESOM, W. H. 1921, 1922 Die van der Waalschen Kohäsionskräfte. *Phys. Ztschr.* 22: 129–141, 643–644 (1921); Die Berechnung der molekularen Quadrupolmomente aus der Zustandsgleichung. *Phys. Ztschr.* 23: 225–228 (1922).

(3) ROSS, C. S. 1924 A method of preparing thin sections of friable rocks. *Amer. Jour. Sci.* 7: 483.

(4) ROSS, C. S. 1926 Method of preparation of sedimentary materials for study. *Econ. Geol.* 21: 454–468.

(5) SCHNEIDERHÖHN, H. 1922 Anleitung zur mikroskopischen Bestimmung und Untersuchung von Erzen und Aufbereitungsprodukten besonders in auffallenden Licht. Berlin.

(6) SHORT, M. N. 1926 The preparation of polished sections of ores. *Econ. Geol.* 21: 648–664.
(7) SHORT, M. N. 1931 Microscopic determination of the ore minerals. *U. S. Geol. Survey Bul.* 825: 10–17.
(8) SIDERI, D. I. 1936 Soil swelling: I. Swelling of soil in water considered in connection with the problem of soil structure. *Soil Sci.* 41: 135–151.
(9) SIDERI, D. I. 1936 On the formation of structure in soil: II. Synthesis of aggregates; on the bonds uniting clay with sand and clay with humus. *Soil Sci.* 42: 461–480.
(10) SIDERI, D. I. 1938 On the formation of structure in soil: IV. The structure of mixed clay-sand and clay-humus formations. *Soil Sci.* 46: 129–137.
(11) SIDERI, D. I. 1938 On the formation of structure in soil: V. Granular structure. *Soil Sci.* 46: 267–271.
(12) SIDERI, D. I. On the analysis of soil structure by the use of the colloid-chemical methods of separation [Russian]. In print.
(13) TYULIN, A. T. 1929 Soil structure. III. Perm. Agr. Exp. Sta. Rpt. No. 3 [Russian].
(14) VEEN, R. W. VAN DER 1925 Mineragraphy and Ore-Deposition, vol. 1. G. Naeff, The Hague.
(15) VLADYCHENSKY, S. A. Report on the work of 1936, Kuznetzk Experiment Station (?). [Russian.]
(16) WHITEHEAD, W. L. 1917 Notes on the technic of mineragraphy. *Econ. Geol.* 12: 697–716.

PLATE 5

FIG. 1. The polished clay section "volkonskonite." Magnification × 20.

FIG. 2. The structure of lamina of desiccated gel (Na-clay "Toida"). Magnification × 20.

FIG. 3. The polished section of "red clay." Magnification × 20.

FIG. 4. Cross section of the clay lamina ("Toida") and the adhesive material (Na-peat humate). Magnification × 23.

FIG. 5. Watertight aggregate of the granular structure of weakly leached chernozem. Magnification × 23.

FIG. 6. Watertight columnar alkali soil. Magnification × 23.

FIG. 7. Watertight aggregate of a podzol soil. Magnification × 23.

FIG. 8. The plowed layer of the soil from the Kuznetzk Experiment Station. Magnification × 23.

FIG. 9. The underlying layer of the soil shown in figure 8. Magnification × 23.

FIG. 10. The chernozem soil (Kamennaya steppe) saturated with sodium and slightly washed with water. Magnification × 23.

FIG. 11. The soil shown in figure 10, between Nicol prisms. Magnification × 23.

FIG. 12. The columnar alkali soil from Volchia River. The vertical section. Magnification × 23.

FIG. 13. The soil shown in figure 12. The horizontal section. Magnification × 23.

FIG. 14. The soil shown in figure 12, between Nicol prisms. Magnification × 23.

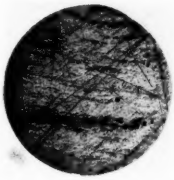


FIG. 1

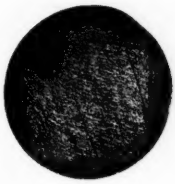


FIG. 2

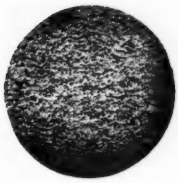


FIG. 3

FIG. 4

FIG. 5

FIG. 6

FIG. 7

FIG. 8

FIG. 9

FIG. 10

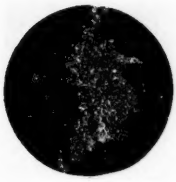


FIG. 11

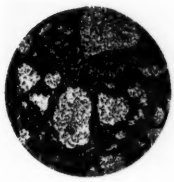


FIG. 12

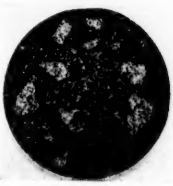


FIG. 13

FIG. 14

PLATE 6

FIG. 1. The serozem soil from the Ak-Kawak Experiment Station. Magnification × 23.

FIG. 2. The soil shown in figure 1, treated with the products of autolysis of *Aspergillus niger*. Magnification × 23.

FIG. 3. The Plavsky chernozem soil. Magnification × 23.

FIG. 4. The Plavsky chernozem covered with a thin layer of autolytic products of *Aspergillus niger*. Magnification × 23.

FIG. 5. The Plavsky chernozem covered with a thick layer of autolytic products of *Aspergillus niger*. Magnification × 23.

FIG. 6. The chernozem soil of Suma Agricultural Experiment Station. Control experiment. Magnification × 20.

FIG. 7. The soil shown in figure 6. With 52 tons of manure. Magnification × 20.

FIG. 8. The soil shown in figure 6. With mineral fertilizers. Magnification × 20.

FIG. 9. The soil shown in figure 6. With 52 tons of manure in a pea-sugar beet crop rotation. Magnification × 20.

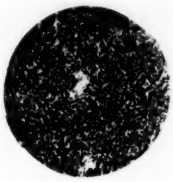


FIG. 1

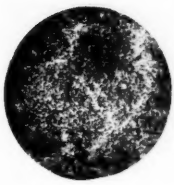


FIG. 2

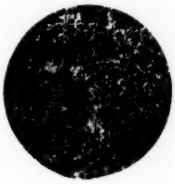


FIG. 3

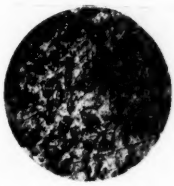


FIG. 4

FIG. 5

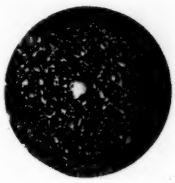


FIG. 6

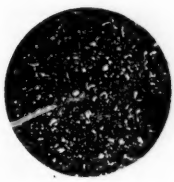


FIG. 7

FIG. 8

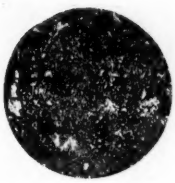


FIG. 9